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VARIATION OF NON-PROTEIN-NITROGEN VALUES IN HEAT-TREATED MUSCLE TISSUES

by

Robert Sayles Yare

A Dissertation Submitted to the
Graduate Faculty in Partial Fulfillment of
The Requirements for the Degree of
DOCTOR OF PHILOSOPHY

Major Subject: Food Technology (Chemistry)

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1952

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I. INTRODUCTION

Systematic research on the biochemistry of muscle began in 1859 when Kühne attempted to relate the clotting of muscle plasma with changes which occur during rigor mortis. Since that time, the increase in the fundamental knowledge of the chemical properties and behavior of muscle has been extremely rapid. While much has been learned relative to the protein structure of meat, there has been too little application of biochemical principles to the technology of meat problems and practice.

A slight advance has been made in the knowledge of the biochemical alterations in proteins which occur in the prolonged storage of meat. However, there has been no progress in the knowledge of the biochemical alteration of meat due to the effect of thermal processing treatments.

Canned meat items, especially beef, have certain palatability defects. Those which are considered to be the most serious are the so-called "canned meat" flavor and the "mushy" texture, the latter being the more objectionable. It would appear that this condition may result from the degradation of muscle proteins. This decomposition probably occurs from the effect of heat, or from the action of naturally occurring proteolytic enzymes (autolysis).

Therefore, this study was undertaken to determine and characterize some of the chemical changes occurring in meat proteins during thermal processing.

II. HISTORICAL REVIEW

Most of the literature regarding the effect of thermal processing on canned meat is devoted to the microbiological phase of the problem. Since this investigation is concerned with chemical changes in meat proteins during processing, a brief background covering many aspects of the biochemistry of muscle appears necessary. Therefore, the following review of literature summarizes the investigations on the composition and characteristics of muscle.

A. The Proteins of Skeletal Muscle

The chemistry of skeletal muscle dates back to 1820 when Bracconet isolated "sucre de gelatin". The first analysis of elastin was made by Tilanus in 1844. Systematic research on muscle and muscle proteins started in 1859 when Kühne reported the relationship between the clotting of muscle plasma and rigor mortis. This same year he reported the isolation of histidine, and the results of a study on the nature of tissue (muscle) autolysis. Kühne called the clot of muscle plasma myosin. Mülder gave the name "protein" to this class of nitrogenous substances. The latter was especially active in studying the chemistry of gelatin, as also were Liebig, Berzelius, and Siegfried. The latter worker

first postulated the reticulin hypothesis of tendon structure.

Because connective tissue is so easily obtained, it has been used extensively by workers to study the chemical properties of the natural tissue proteins, collagen and elastin, and the derived protein, gelatin. The early efforts of Abderhalden and Hoppe-Seyler in Germany, Lloyd and Marriot in England, Ames in Scotland, and Chittenden, Gies, Niemann, Chibnall, Bouge, and Thies in this country have resulted in a great wealth of information on the chemical reactions and structure of collagen, elastin, and gelatin.

Danielewsky, von Fürth, and Halliburton were much influenced by Kuhne's work in their attempts to explain the *in vitro* properties of muscle proteins (other than those from connective tissue) in terms of the behavior of muscle itself. Danielewsky (1) prepared myosin by extracting muscle with 10 to 15 per cent ammonium chloride, and subsequently precipitating the protein by dilution with water. von Fürth (2,3) by extracting rabbit muscle with 0.6 per cent sodium chloride, isolated a globulin which he called myosin, and another protein, myogen. As the solvent was inadequate for completely dissolving the globulin, the yield of myosin was low. However, he did observe carefully the quantities of neutral salts necessary to salt-out myosin. In addition, he studied the precipitation of the protein on

dialysis and by low concentrations of acetic or mineral acids, and also noted its solubility in an excess of acid. Halliburton's (4) paramyosinogen appears to be identical with the myosin of Danielewsky and von Fürth with the exception that he found no precipitation of the protein in the presence of acetic acid. von Fürth explained this on the basis that Halliburton had added an excess of acid in which the protein is soluble.

The first extensive fractionation of muscle was performed by Howe (5). Using monopotassium phosphate-dipotassium phosphate mixtures, he obtained five fractions; paramyosinogen, myosinogen, myoglobulin, total globulin, and albumin. In this paper he attempted to clarify the confusion which then existed relative to the properties and the nomenclature for muscle proteins.

Weber (6) and Edsall (7) isolated myosin. The latter studied the physicochemical properties of the protein. The methods of preparation and purification described by Edsall keep only the myosin in solution and so yield preparations free from other muscle proteins.

In 1933 Weber and Meyer (8) suggested the existance of a third protein in muscle plasma; it was named "Globulin X". The evidence for the existance of this protein was based on an examination of the precipitate formed during the dialysis

of muscle-press juice and salt extracts. The separation from myosin, which behaves similarly, was made on the basis of the difference in solubility in certain salt solutions.

Bate-Smith (9) undertook a study of muscle proteins, the object of which was twofold; (1), to determine whether the denaturation products or complexes formed between native and denatured proteins had been mistaken for new native proteins, and (2), to investigate the possibility of improving the fractionation techniques of obtaining purer compounds for chemical and physicochemical studies. As a result of these investigations, the existance of Globulin X was confirmed, and, in addition, evidence was presented for the existance of a fourth native protein. This latter protein had the properties of an albumin and was named myoalbumin. It differed from myogen (also an albumin) by its low isoelectric point, its solubility at high salt concentrations, and its behavior when treated with dilute acids. In regard to improving fractionation procedures, Bate-Smith stated that there was every reason to believe that myosin, as ordinarily prepared, consists of a single protein species; that myogen can only be separated from myoalbumin in the denatured state; and that globulin X was the most difficult fraction to prepare because of the tendency of denatured proteins to disperse in its solutions. He showed that the four fractions

differed with respect to their isoelectric point, solubility in water, and in the salt solutions of both native and denatured proteins.

In recent years, progress has been made in the purification of proteins from the sarcoplasma (muscle fibrils). In 1939 Baranowski (10,11) isolated two crystalline proteins from the myogen of rabbit muscle; myogen A, occurred in the form of hexagonal bipyramids, and myogen B, as thin transparent crystals. Other researchers have shown myogen A to be homogeneous in the ultracentrifuge, and have found that it possesses aldolase-zymohexase activity. No physiological properties have as yet been ascribed to myogen B.

More recently Szent-Györgyi (12) has demonstrated that the main constitutent of the muscle fibril is myosin of which there are two types: liquid myosin A and viscous myosin B. The change of myosin from type A to B is attributed to the slow extraction of a third protein actin. The latter has the striking property of existing in a globular as well as fibrous form. It can be extracted from muscle only after it has been liberated from its bonds and depolymerized into globules. G-actin, discovered by F. B. Straub in Szent-Györgyi's laboratory, is a typical globular protein.

Bailey (13) and Disteche (14) are among the latest

workers to announce the isolation of proteins from skeletal muscle. Bailey describes his tropomyosin as a homogeneous, asymmetric protein that constitutes about 0.5 per cent of wet, skeletal muscle of the rabbit. Disteche's protein, belonging to the albumin class and possessing a globular form, was precipitated by the addition of ammonium sulfate to 80 per cent saturation, from the soluble fraction obtained after treatment of the aqueous muscle extract with 0.05 volumes of hydrochloric acid and heating to 90° C.

1. Post mortem changes in muscle

Living muscle has a pH in the neighborhood of 7.4 according to Voegtlin and co-workers (15). When the blood supply to the muscle is stopped, its characteristic, highly reversible reactions are interrupted. Immediately the pH of the muscle falls. In addition, there is a depletion of the substrates necessary for living functions and an accumulation of the end products of autolytic reactions. Carbon dioxide is liberated by the destruction of bicarbonate ions, creatine phosphate is broken down; the proteins lose about two-thirds of their buffering capacity. The substances which subsequently act as buffers are phosphate compounds, proteins, carnosine and anserine. Concurrent with the change in pH, caused by the conversion of glycogen into lactic acid, there

is a change in the physical state of the muscle. The muscle stiffens or sets (rigor mortis).

Fletcher and Hopkins (17) regarded the fall in pH as the cause of rigor mortis. It is now known that a change in pH alters the physical properties of the muscle proteins, particularly myosin, and causes a shrinkage of the fibrils, to which the change in color, the ease of expression of juice, and the easier penetration of salts during rigor are attributable. However, as early as 1877, Claude Bernard (18) noted that rigor mortis occurred even in muscles poor in glycogen. Best et al. (19) and Hoet and Marks (20) have made similar observations by producing rigor in animals made hypoglycemic by the administration of insulin or the feeding of thyroid extract. The one fact that rules out lactic acid as the cause of rigor mortis was demonstrated by Ronzoni (21) with muscle poisoned with sodium iodoacetate, a situation which blocks the breakdown of glycogen to lactic acid, the muscle passed into rigor in a state of contracture.

Bate-Smith (22) observed a curious phenomenon. He noted that when enough acid is produced to lower the pH of the muscle to approximately 6.3 rigor always develops. A possible explanation of this was published later by Bate-Smith and Bendall (23). They suggested that the connecting link is the mineralizing phosphatase of Sakov, which produces inorganic

phosphate from adenosinetriphosphate. This enzyme which is comparatively inactive at the pH of living muscle, becomes increasingly more active at pH 6.5 and below. Erdos (24) had shown that adenosinetriphosphate disappeared from muscle with the beginning of rigor mortis, and Bate-Smith and Bendall, confirming this, concluded that the immediate cause of the stiffening of muscle in rigor mortis is the removal of adenosinetriphosphate. As the pH of muscle falls to below pH 6.15, the adenosinetriphosphate is destroyed by the action of the enzyme to the extent that rigor develops. Bate-Smith states that the connection between rigor and pH is reasonably explained but raises the question as to how the physical condition of muscle can be so greatly altered by the removal of one component.

Hoet, previously mentioned, postulated that the blocking of the reaction between glycogen and phosphate to form hexosephosphate is necessary for the onset of rigor. Szent-Györgyi (25) and Bate-Smith (26) have recently advanced new theories to account for the development of rigor mortis. Szent-Györgyi's theory is based on the presence of myosin, actin, adenosinetriphosphate, and the potassium, calcium, and magnesium ions in muscle. In muscles at rest, Myosin A, complexed with the ions present and the adenosinetriphosphate, is combined with the fibrous form (F) of actin. When the

muscle is stimulated to perform work, the complex is decomposed to displace some of the potassium and adenosinetriphosphate. Because of the loss of this ion, F-actin combines with myosin A. When this latter combination occurs, actomyosin, in the presence of adenosinetriphosphate, contracts, the adenosinetriphosphate having been furnished by the original stimulation. When the free adenosinetriphosphate is removed by the enzyme function associated with contracted actomyosin, the system reverts to that necessary for the resting condition. The muscle at rest is extensible because the myosin A is in globular form and not associated with actin. If potassium ions (by diffusion) and adenosinetriphosphate (by enzymic action) are removed from myosin A, as occurs when the muscle dies, actin combines with myosin A to form actomyosin. Actomyosin, which must exist in the extended form in the absence of adenosinetriphosphate, is inextensible and confers on dead muscle the characteristic rigidity of rigor mortis. Bate-Smith's theory is based on certain physical and chemical properties of muscle which include:

a. The extension-time curve on loading a resting muscle differs from that for a stimulated one.

b. A muscle does not usually contract when it passes into rigor and the form of its extension-time curve is characteristic of that of resting muscle.

c. There is a decrease in the amount of extractable proteins when a muscle is in rigor, although the solubility of fibrillar protein is not changed.

d. There is little or no change in the diameter of the particles present in the ultimate filaments of the muscle fiber and those in a solution of myosin extracted during rigor. A particle represents a number of myosin molecules packed side by side.

e. Adenosinetriphosphate has the property of decreasing the viscosity and double refraction of flow of myosin solutions _in vitro_, increasing the amount of myosin extracted from muscles in rigor, increasing the extensibility of myosin threads, and shortening actomyosin threads. The disappearance of adenosinetriphosphate, the decrease in the extractibility of proteins, and the stiffening of muscle during rigor are somehow related.

From these facts Bate-Smith deduces first, that the particles observed in myosin solutions are fragments of the ultimate contractile filaments, and that these are not necessarily composed of homogeneous molecules; second, these filaments are associated thru cross linkages by the process of rigor mortis rendering them more difficultly separable by the action of extractant salts, but, once separated, little difference in

solubility characteristics exist. Cross linkages, accounting for the decrease in extensibility, are formed as a result of the removal of adenosinetriphosphate. Contraction, on the other hand, is attributed to the action of adenosinetriphosphatase of myosin on adenosinetriphosphate. Contraction alters the form of the extension-time curve but not the absolute extensibility.

A close examination of these two theories reveals certain aspects in common. However, Bate-Smith emphasizes interfilamentary reactions during rigor while Szent-Györgyi stresses intimate molecular processes.

Not only are there outward manifestations of rigor but characteristic changes in microscopic structure as well. Paul (27) observed the appearance of rigor nodes at the outset of rigor in the examination of histological sections of beef muscle. The nodes persisted during rigor, its resolution, and subsequent storage of the muscle. These nodes, which are widened areas, alternate with narrow areas along the muscle fiber. In these widened areas, the cross striations of the fibrils are densely packed, while in the alternating areas, the striations are not as distinct. In many instances she observed that contraction in the node had distorted the neighboring fibers into wavy and twisted configurations.

2. Denaturation and coagulation

Proteins undergo characteristic changes known as denaturation. Among the agents which induce this reaction are strong acids or bases, alcohol, sulfonated alcohols, urea, guanidine, x-rays, ultra-violet light, heat, surfaces, forces, and enzymes. Denaturation usually involves the following changes from the native state: (1) decreased solubility, (2) loss of crystallizability, (3) loss of specific biological activity, (4) increased viscosity of solution, (5) change in molecular shape, (6) exposure of oxidizing and reducing groups (sulfhydryl and disulfide linkages), and (7) increased susceptibility to enzymic action.

The early work in protein denaturation was carried out by Chick and Martin and by Wu. In recent years, Mirsky and Pauling (28) have expanded the ideas of Wu into the theory that denaturation is considered to be a change in the highly specific structure exhibited by native proteins to a randomized arrangement of the changed molecules.

The decreased solubility can be accounted for by two changes in the native molecule. Water soluble polar groups unite with other polar groups thru a shift in the specific configuration, and thereafter are not available for water binding. Secondly, the protein molecules become asymmetrical, resulting in greater cohesion between the molecules;

therefore, decreased solubility. This change in symmetry is indicated by a decrease in the solubility of protein solutions upon denaturation.

The sulfhydryl and disulfide groups of proteins have been investigated by Finn, Neurath, Greenstein, Mirsky, Anson, and others. Finn (29) discusses the denaturation of the proteins in muscle juice by freezing at -2° to -3° C. He observed that denaturation reaches a maximum in 25 to 40 days and amounts to about 20 per cent of the total coagulable-nitrogen. The critical pH is 6, below which there is a rapid increase. From 25 to 30 per cent of the total protein is denatured at this temperature in 40 days. Finn claims that one fraction of the proteins is more easily denatured than are the others, probably one part of von Fürth's myogen fraction, which he says can be explained by the variation in pH and changes in salt concentration.

Mirsky (30,31) reported that protein coagulation by denaturing agents is different from coagulation of myosin by the unknown agent in muscle since denaturing agents activated sulfhydryl groups, whereas the formation of insoluble protein by rigor was not accompanied by the activation of these groups. In the second paper, a differentiation is made between the denaturation which occurs in rigor mortis, in dehydration, and in muscular contraction and that which

occurs by the action of acid or heat. The action of heat or acid ruptures disulfide groups so that sulfhydryl groups can then be titrated, but, in the case of the others, none can be detected.

The increase of sulfhydryl groups on denaturation can be demonstrated in such cases as globin, egg albumin, and edestin. In the native state, these proteins give a negative nitroprusside test, but upon denaturation the test becomes positive. Other proteins, myosin and hemoglobin, among others, which have active sulfhydryl groups in the native state, show an increased number upon denaturation.

Sadikov and Starukhina (32) reporting on the thermal denaturing of meat state that when meat is heated at 85°, 95°, 110° and 120° C. the loss in water-soluble protein ranges from 50 to 70 per cent with some loss in amino-nitrogen. Whether the change is reversible dehydration or irreversible denaturation is not clear. They also state that heating meat for two hours at 65° to 100° C. sometimes increases amide-nitrogen by 1 to 2 per cent.

3. <u>Muscle proteins and their relationship to structure and function</u>

In striated muscle, the muscle mass consists of bundles or fasciculi, the bundles are composed of smaller bundles, and

the smaller bundles are made up of fibers. Each fiber is made up of bundles of minute fibrils or sarcostyles embedded in a semi-fluid contractile substance known as the sarcoplasm. The fibrils, long, narrow, and multinucleated, are composed of sarcous elements or sarcomeres, minute bodies of a peculiar structure placed end to end in the fibril-like beads on a string. Fibers are held together by a framework of connective tissue called endomysium and another sheath of connective tissue, the perimysium. The fiber so composed is enclosed in the sarcolemma.

The fibrils, or myofibrils as they are more commonly called, show markings which produce a transversely striated appearance. The fibrils consist of alternating light and dark segments which form the cross striations of the fibers. In a given fiber the dark portions of the fibrils are adjacent, as are the light portions. The dark portions form the anisotropic (A or Q) cross striations, the light portions the isotropic (I or J). It has been shown that in the lighter bands the myosin is more highly folded or is in a less oriented condition, so that the bands cause cross striations, which are more distinct than the longitudinal markings.

The animal body makes use of a number of muscular proteins for structural purposes. However, the number present and the role each one plays in the structure and functioning of a

muscle fiber is the subject of much discussion. Those that are recognized have been discussed previously. They are all fibrous proteins and with the exception of myosin are extracellular. They are all water-insoluble except, again, muscle myosin. Myosin is now recognized as the contractile part of the tissue, while the extracellular proteins of tissue are considered as supporting structural components. Astbury (33) has used X-ray diffraction analysis to study the structure of myosin to explain its role as the contractile element. His analyses show that the molecule, a fibrous protein with a "back-bone" of considerable length, has the ability to fold, and so becomes shorter or contract. The folded form is α -myosin which becomes β -myosin on extension. In its contractility and anisotropy, only the A or anisotropic band of myosin resembles the smooth muscle fiber, while the I or isotropic band appears to be a more extensible structure of unknown function. Some relationship exists between contractility and the chemical reactions occurring in muscles, as yet, obscure, but, supposedly they affect ultimately the ionization of the myosin chain, and hence the length, by (1) a gross change of pH, (2) a localized change in the neighborhood of certain groups, and (3) by esterification of some side-chain groups which previously contributed to the total charge. A gross change in pH is not probable, but the others

are given important consideration in view of the knowledge that a part of myosin is endowed with adenosinetriphosphatase activity. A localized reaction occurring at some grouping could influence the configuration of other groups. This could result in the formation of a co-valent link, or, more probable, in the formation of metastable links between enzyme group and reaction products, as with adenosinetriphosphate. The calcium ion might be an activator made available by the stimulus to set the reaction in motion. Basically this is the older view of muscular contraction, that chemical reactions occur at the same time as, or just prior to, the contraction. It is also possible adenosinetriphosphate takes part in the relaxation process. Needham has formulated such a theory and Brown has proposed a theory similar to those which consider the adenosinetriphosphatase activity of myosin. The presence of calcium and magnesium ions in the refracting band and why adenine nucleotides, located in the isotropic regions in relaxed muscles, diffuses into the anisotropic on fatigue must be explained by any theory set forth.

B. Chemical Studies On Animal Tissue

Fundamental knowledge of the physiology and biochemical properties and behavior of muscle has increased tremendously

since the time of Kühne. While the advancement in this aspect of muscle chemistry has been tremendous, there has been no such advancement in the fundamental knowledge of the biochemical processes involved in the "aging" of animal tissue, nor any extensive application of the biochemistry of muscle to the technology of handling of meat animals and meat. While many investigators have sought the answer to the question of what happens chemically during the "aging" or "ripening" of meat during storage, the riddle remains essentially unsolved.

Lehmann (34) was the first to attack the problem of aging when in 1907 he reported the results of ten year's work. Both physical and chemical analyses were used to measure the changes in tenderness in two beef muscles, the psoas (tender) and flank skin muscle (tough), on storage. He devised the dexometer, a mechanical device which determined the force required to bite through a sample, and another instrument which measured the breaking strength of the muscle. Using the dexometer, he found the flank muscle to be 2.4 times tougher than the psoas. Histological studies of the muscle fibers showed those of older animals to be approximately two and one-half times as thick as those of young animals. Collagen analysis showed the psoas to contain 0.3 to 0.5 per cent and the flank muscle to have 0.8

to 1.4 per cent. On storing the muscles for eight days, he noted a tenderizing effect with aging, more noticeable in the case of the flank muscle. The same trend was evident, but not as striking, in the case of meat boiled five, thirty, or sixty minutes. He observed that freezing and thawing lessened the toughness of muscle, aged muscle showing the greater change. Lehmann also conducted experimental work on the cooking of meat. He showed that although in the raw state the flank muscle was 2.63 times as tough as the psoas, after boiling for one to one and one-half hours they were equal; after three hours the flank muscle was more tender than the psoas. The psoas may have been slightly tougher after a short period of cooking than it was in the raw state. He explained this by saying that the coagulation of "albumins" and the loss of elasticity made the cooked muscle difficult to cut. Opposing this was the tenderizing of connective tissue. If this tenderization did not balance the coagulation or loss of elasticity, the cooked meat was tougher. Considering the time at which the work was done and the extent of the development of muscle chemistry, it must be said that this phase of the study was an outstanding example of the high quality of Lehmann's work.

At about the same time, Grindley and Emmet (35) at the University of Illinois carried out and published the results

of a series of studies entitled, "The Chemistry of Flesh". The determinations they made are of little value today because the distinction between coagulable and non-coagulable protein is no longer of much significance.

As early as 1910, Meyers (36) considered autolytic changes of primary importance and bacterial products secondary in the ripening of meat. Although most of the studies to date on the physiology of meat ripening have approached the problem from the autolytic standpoint, there are those workers who consider the increase in tenderness during aging as resulting from the action of bacteria.

In 1917 Hoagland, McBride and Powick (37) published the results of one of the most extensive studies on post mortem changes in beef ever undertaken. They followed the change in pH, non-coagulable nitrogen, proteose-nitrogen, amino-nitrogen, ammonical-nitrogen, soluble organic phosphorus, and the pH of the kidney and external fat in seven quarters of beef held in cold storage just above the freezing point for a period of time varying from fourteen to one hundred and seventy-seven days. They found the same changes taking place as had been noted when beef was autolyzed under aseptic conditions for seven to one hundred days. This eliminated bacteria as the cause of the changes during aging and supported the earlier work of Meyers. The coagulable-

nitrogen decreased at about the same rate that the non-coagulable-nitrogen increased. The amino-nitrogen and ammonia-nitrogen also increased, but in the case of the latter, the rate decreased as it accumulated. The total soluble phosphorus varied irregularily, but that fraction of the total which was inorganic increased. The increase in tenderness accompanying aging was complete in two to four weeks. This work was well planned and executed, but the fractionation of the nitrogen into the nondescript coagulable and non-coagulable forms makes it all but impossible to compare the results with those studies using the more refined techniques of recent years.

In 1922 Fearon and Foster (38) published a study on the effects of freezing and thawing on the quality of beef. They concluded that beef cannot be frozen and thawed without marked changes taking place in the appearance, palatability, and general physical condition of the meat. Two years later, Foster (39) presented a report at the Fourth International Congress of Refrigeration which contained the results of chemical investigations on the autolysis of beef. The soluble-nitrogen content of the total nitrogen of aging beef increased from 10.5 per cent to 13 per cent in ten days; thereafter, it remained constant. Freezing beef at -8° C. increased the soluble-nitrogen from 10.5 per cent to

16 per cent. In this study trichloroacetic acid was used to precipitate the protein-nitrogen. These results seem to indicate a greater autolysis due to freezing; this is contrary to present knowledge.

Reay (40), in England, using haddock as the test species, concluded that the change during storage affects the colloidal state of the muscle globulin. According to this worker, the muscle globulin denatures and loses some of its power to hold water. The denaturation results from the action of the concentrated salt solution in the partly frozen muscle.

In 1924, Tressler, Birdseye, and Murry (41) reported that quick freezing beef and subsequent frozen storage effects a marked tenderizing.

Finn (29) has discussed the effect of freezing at -2° to -3° C. on the denaturation of muscle protein (see page 15). He concluded that if there is an intimate relationship between "drip" and denaturation, it would appear that the former could be reduced by keeping the pH in the frozen state at or above 6, the critical pH for denaturation in the frozen state.

Many investigators have attempted to explain quality of beef in terms of chemical characteristics. Their approach to the problem is a more practical one. For example, Noble and co-workers (42), measuring tenderness and juiciness in beef,

found a relationship between juice, total-nitrogen, coagulable-nitrogen, and juice solids. They stated that the more juice in the beef, the higher it is in solids, total nitrogen, and, in one instance, coagulable-nitrogen. Baker (43), recognizing that little fundamental work had been done on meat proteins since the time of von Fürth and Halliburton, concluded that the following constituents of meat act as indicators of change: (1) lactic acid content, (2) pH, (3) moisture, proteins, and extractives, (4) hemoglobin and color, (5) ammonia-nitrogen, and (6) free fatty acids. Mackintosh, Hall, and Vail (44) and Brady (45) contend that tenderness is a function of texture. Mackintosh and his co-workers state that the higher the collagen-nitrogen, the less tender the meat, the higher the shear value, and the lower the palatability. Hammond (46) also contends tenderness is a function of texture with texture, in turn, depending on the size of the muscle fiber. This is in disagreement with Mackintosh, who believes that connective tissue is the contributing factor.

At this same time, a group of Russian researchers were engaged in the study of the aging of meat. They studied changes in proteins, protein decomposition as determined by the appearance of protein decomposition products, and changes resulting from enzymic activity during aging. Smorodintsev

and Kuilova (47) found that neither fresh beef muscle nor that stored up to fifteen days at 1° C. contained albumoses or peptones. This finding would seem to indicate that muscle proteins remained intact during storage, or if decomposition occurred, it progressed beyond a point where molecules of intermediate size are formed. Smorodintsev (48,49) considered the maturing of meat to be linked with the conversion to the coagulated state of part of the active proteins of muscle plasma, "active albuminoids", that are liquid when the animal is living. His classification of a protein fraction as an "active albuminoid" seems confusing. Nevertheless, this theory, which considers the aging of meat to involve a denaturation or coagulation of a portion of the muscle proteins rather than a disintegration, is based on chemical evidence gathered by Smorodintsev, Krylova, and Pasonina (50). They found that the non-protein-nitrogen level remained practically constant at 0.32 to 0.34 per cent. In addition these authors found that the ratio of the stroma protein-nitrogen (the protein fraction insoluble in 5 per cent sodium chloride) to the salt extracted nitrogen (the fraction soluble in 5 per cent salt and consisting of myosin and non-protein extractives) was 0.89 one hour after killing, 2.38 after storage for twenty-four hours, 2.28 after seventy-two hours storage, and 2.10 after one hundred and twenty hours of storage. They

concluded that no marked disintegration of protein occurred and that part of the myosin changed to an insoluble form. This work and the theory based upon it can be criticized from one point. The study lasted only one hundred and twenty hours. The changes occurring in this time cannot be questioned, but meat is usually considered to require at least fifteen days for proper aging. Would these same changes have been the major ones if storage had continued for a longer period of time or would other changes, possibly pointing to a disintegration by enzymic action have been observed? If so, they may have considered aging to be autolytic in nature. That this is possible is evident from additional work done by Smorodintsev which will be reviewed in a later section on autolysis of muscle.

Drozdov and Drozdova (51) studied the biochemical changes in muscle tissue on freezing. They observed an increase in acidity, as measured by a lowering of the pH and an increase in lactic acid, in glucose, and in acid-soluble and inorganic phosphorus. Also, there was some increase in protein-nitrogen, but residual and amino-nitrogen remained unchanged.

Callow (52, 53, 54), in England, has studied the relationship between pH and electrical resistance and the incidence of "dark cutting" beef. Shortly after slaughter the

the electrical resistance (measured with a Banfield probe in conjunction with a "Megger" Earth Tester at 50 cycles) was 1700 to 2000 ohms across the fibers and approximately 1200 ohms along the fibers. As rigor developed, both values fell, usually reaching a value of 220 to 400 ohms and the difference between the two measurements became less marked. The correlation between increasing pH and increased resistance was considered as being related to swelling of the fibers, which causes a narrowing of the channels through which electrical forces can exert an influence. Callow terms this condition a "closed" structure as opposed to an "open" structure observed at lower pH values. The "open" structure is associated with a moist feel, firm texture, and pale color; the "closed" structure with a dry feel, flabby texture, and a deeper color. A stickiness is also noted in the latter case [which according to Bate-Smith (55) is not due so much to a swelling of the fibers as it is due to a certain degree of actual dissolution of myosin]. Hall and co-workers (56), in his work at the Kansas Agricultural Experiment Station, has shown that the "openness" or "closeness" of the structure is not the only possible cause of the dark color. He found that the demand for oxygen in "dark" beef is greater than can be obtained by transfusion through the tissue, and consequently, oxyhemoglobin is forced to release some of its oxygen.

Steiner (57), reporting on changes in mechanical strength in beef muscle during aging at 0°, 5°, 10°, and 23° C., concluded that fresh meat decreases in toughness on aging because the muscle fibers become inelastic and brittle. The mechanical properties of the connective tissue do not change. These conclusions suggest a change in the structure of the tissue proteins.

In 1942, Weidlein at the Mellon Institute, described a "Tender-Ray" process for the accelerated aging of beef. In this process, beef carcasses are held at 60° F. for forty-eight hours in the presence of ultra violet radiation. It was claimed that aging under these conditions produced the same result as a thirty day ripening period at 35° F. In comparing the rate of chemical change between these two processes, McCarthy and King (58) found a more rapid increase in sulfhydryl groups and in soluble-nitrogen during the accelerated process.

Ramsbottom, Strandine, and Koonz (59) compared the tenderness of twenty five beef muscles, both raw and cooked. Their primary interest lay in the tenderness of the cooked samples rather than in the raw ones. They concluded that cooked muscles decreased in tenderness due to the coagulation and denaturation of muscle proteins and the shrinking and hardening of muscle fibers.

According to Deatherage and Harsham (60) the development of tenderness in beef does not proceed in a smooth curvilinear fashion with time at 33° to 35° C. At seventeen days there was a break in the tenderness value curve in many cases, the value being lower at twenty days, but improving somewhat by the thirty-first day. These workers postulated a theory in which muscle plasma chemistry is a major factor in the tenderness of beef.

Husaini et al. (61, 62) recently published the results of an extensive study on the biochemistry of beef as related to tenderness. In their first study they found a close negative correlation between tenderness and alkali-insoluble proteins (collagen and elastin), no correlation between tenderness scores and total-nitrogen, trichloro-acetic-acid-soluble-nitrogen, a low but significant correlation between marbling and tenderness, and no relation between tenderness and pH, moisture, lactic acid, or inorganic phosphate. In a second study they dealt with the relationship of biochemical factors to changes in tenderness during storage. Tenderness was determined at three and fifteen days. In all cases, tenderness increased. In the cases of eight out of twenty eight beef carcasses, there was no significant change in the nitrogen soluble in trichloroacetic acid and zinc hydroxide between three and fifteen days post

mortem. From this observation they concluded that protein changes responsible for increased tenderness on aging were too subtle to be determined. At fifteen days they observed a correlation between tenderness and alkali-insoluble proteins; however it was not as high as previously reported.

Muscle hemoglobin showed no relation to tenderness at three days, but a very significant correlation was found between the third and fifteenth day. These observations were interpreted as indicating the importance of muscle plasma in the tenderness of meat. They stated that it appears that both connective tissue (as represented by alkali-insoluble protein) and muscle plasma (as represented by muscle hemoglobin) are in part responsible for tenderness in meat. However, there was no indication of the fundamental relationship between muscle hemoglobin and tenderness other than the fact that the amount of muscle hemoglobin is directly related to the muscle plasma and its function.

C. Autolytic Studies on Muscle

Salkowski (63) in 1890 called attention to the fact that animal organs when kept at 37° C. with an antiseptic undergo a change more or less similar to the digestion of protein in the alimentary tract. Soon after it was observed that hydrochloric acid has a favorable influence on autolysis.

Although not recognized as such, the dependence of autolytic activity on pH was established. Whereas most of the studies on autolysis have used organs (e.g., liver, spleen) as the test material, there have been published the results of some studies on the autolysis of striated muscle.

Chen and Bradley (64) studied the autolysis of many different muscles, including beef and the dark and light muscle of chicken. They found that pigmented tissue autolyzed to a greater extent than tissue not pigmented and that beef muscle autolyzed to a greater extent than the dark muscle of chicken. The incubation mixture used contained 50 grams of tissue in 500 ml. total volume, with varying volumes of N/5 hydrochloric acid added to adjust the pH. The degree of autolysis was determined by measuring the production of amino acids; the latter were obtained from the filtrate after precipitation of the tissue proteins with trichloroacetic acid. They concluded that muscle tissue from warm blooded animals autolyzes under much the same conditions as glandular tissue, but does not digest to the same extent. They stated that this difference is due to the greater connective tissue content of muscle, and also to the presence within the muscle cell of structural proteins which are not affected by the cell proteases under any conditions. They contend that only a small fraction of the total muscle proteins are

susceptible to atrophic hydrolysis, and that if the structural proteins digest, it must be an extremely slow process or it may involve other enzymes than those found in muscle (phagocytic cells).

For many years, autolytic studies were made using the whole organ or muscle, or at best a crude preparation from them on some other substrate. However, in 1929, Willstätter and Bamann (65) announced the isolation of an intracellular proteolytic enzyme from gastric mucosa. Later Waldschmidt-Leitz and co-workers (66) concluded from the action on gelatin and certain synthetic di- and tri-peptides that liver and spleen extracts contain four types of proteolytic enzymes; a proteinase acting at a weakly acid pH (Willstätter's "cathepsin"), a catheptic carboxypeptidase, an aminopeptidase, and a dipeptidase. These have been named cathepsins I, II, III, and IV. Their chemical nature and specificity have been extensively investigated.

With the discovery of the cathepsins, attempts were made to determine the role they played in meat ripening. These investigations were not necessarily confined to this group of proteolytic enzymes but to the role played by other intracellular proteinases as well. For example, Sadikov and Shoshin (67) reported that at 17° C. there is a slight alteration in globulin in two days, and marked alteration in twenty

seven days. At 37° C., there is extensive peptone formation up to four months and at 55° to 60° C. the degradation of meat proceeds in a few days to amino acids. They concluded that ripening of stored meats is apparently not autolytic but a process equivalent to enzyme stabilization.

Smorodintsev (68) found that cathepsin played only a minor role in meat ripening. During the autolysis of beef at 1° to 4° C., cathepsin activity decreased 40 to 45 per cent in the first twenty-four hours. During the next five days the activity decreased 20 per cent. On the other hand, he found (69) that the activity of a water-soluble peptidase increased two and one half times that found at the time of slaughter when beef was stored at 3° C. for ten days.

Balls (70), in a review of enzymic action in food products at low temperatures, states that the role of cathepsin in meat ripening is not known. It is evident from the papers reviewed on the subject that this is the case.

D. Investigations on Canned Beef

The investigations on canned beef have been directed primarily to studies on the bactericidal effects of heat processing. Consequently, little fundamental knowledge is available concerning chemical changes which may occur.

In Japan, Okuda and Yamafugi (71) studied changes in

muscle protein during canning. Samples prepared from chicken, whale, and fish, were sealed in tubes and heated at 140° C. for one hour. About 20 per cent of the protein was hydrolyzed. They observed that the quantities of cystine, arginine, histidine, and lysine decreased, that the change in the quantities of tryptophane and tyrosine were small, and that the quantity of proline remained unchanged during heat processing. Protein-sulfur was converted into hydrogen sulfide, sulfide-sulfur, sulfate-sulfur, and soluble organic-sulfur when the tissue was alkaline. They also noted that at higher pH values more ammonia and hydrogen sulfide formed. In 1938, Okuda (72) published the results of additional work on canning. He observed much the same effects as he had previously. In addition, he noted that the pH became nearer neutral with processing, and that total-nitrogen, cystine-nitrogen, and ammonia-nitrogen decreased while melanine-nitrogen increased.

Green (73), processed beef in No. 2 cans at a temperature of 240° F. for sixty five and ninety minutes and compared the effects of sodium chloride, lactic acid, and sodium chloride plus lactic acid injections on the palatability of the finished product. In addition she studied the effect of processing times at a processing temperature of 240° F. Her results indicated that processing for ninety minutes produced a canned product with a lower aroma score than a

sixty five minute process, but that the longer processing increased the tenderness of the meat slightly. Injection also increased tenderness. Sodium chloride increased the juiciness of meat processed for sixty five minutes but not that processed for the longer time. She also observed that the pH of the meat became more alkaline with processing.

III. EXPERIMENTAL INVESTIGATION

This problem was undertaken on the hypothesis that proteolysis due to an increased proteolytic activity during the initial stages of thermal processing, in addition to chemical hydrolysis of the meat proteins during processing, may be in part responsible for the "mushy" texture encountered in canned meat items. These proteolytic changes may be somehow related to those post mortem changes that result in an increase in tenderness during storage.

With chicken breast muscle (pectoralis major) as the test material, the change which is observed as a result of storage is an increase in nitrogen not precipitated by protein precipitants (non-protein-nitrogen). This increase has been interpreted as due to a breakdown of protein constituents. Therefore, it seemed desirable to study this breakdown of protein material by incubating similar tissue samples under autolyzing conditions. Such conditions would increase the rate of activity of the naturally occurring proteolytic enzymes which supposedly cause this increase in non-protein-nitrogen under normal storage conditions. Thus, by artificially exaggerating this effect, it was expected that the nature of the proteolysis which occurs in storage might be identified from a chemical standpoint and correlated

with changes accompanying the tenderization due to storage.

The data from the incubation studies should show a correlation with that for the natural proteolytic activity of meat. The accelerated activity of the proteolytic enzymes, as tissue samples are incubated, may be related and perhaps even identical with the activity which is encountered in the exposure to heat which occurs in canning and other types of heat processing. As canned meat is exposed to processing there is a marked increase in temperature over time. In the time interval prior to attaining the maximum temperature, enzymic activity of the tissue may be greatly accelerated. At the maximum temperature attained in the can the enzymes may be presumed to be inactivated and further proteolysis thereby stopped. Therefore, a study of this nature would help to clarify the situation in regard to changes produced in storage, and also to the chemical reactions which occur in heat processing and canning.

A. Autolysis Studies

The autolysis studies were undertaken in an attempt to characterize the breakdown or hydrolysis of protein tissue by naturally occurring proteolytic enzymes under accelerated conditions. It was decided that the breakdown could be followed by determining the increase in non-protein-nitrogen.

The nature of the decomposition could then be defined by the decomposition products formed. The type of decomposition products formed could be determined by analyzing, for nitrogen, deproteinized filtrates from incubated muscle suspensions.

1. Basis of methods chosen

According to Hiller and van Slyke (74) trichloroacetic acid, especially in concentrations of 5 per cent and below, removes from protein suspensions only protein and leaves in solution intermediate products of protein decomposition (proteoses and peptones), the lower peptides, and amino acids. One per cent tungstic acid, on the other hand, precipitates not only protein, but intermediate products as well, and leaves in solution only lower peptides and amino acids. Thus, by using these two precipitants, it is possible to determine whether the degradation ruptures the proteins to produce molecules of intermediate size, or splits off small fragments (lower peptides and amino acids), leaving most of the protein molecule intact.

2. Preparation of incubation suspensions

Chicken breast muscle (pectaloris major) was used as the test material. Live chickens, obtained from the College

Poultry Farm, were slaughtered by severing the jugular vein and electrocuting (110 V AC) for ten seconds. After bleeding had nearly ceased, the bird was agitated in water at 130° F. for ninety seconds, then defeathered. The skin covering the breast was split open, and the right and left pectaloris major muscles were removed. The muscles were ground by passing through a food chopper three times, and then thoroughly mixed to insure uniformity in sampling. Two duplicate samples of 25 grams each were weighed out on a torsion balance. Each sample was homogenized with 100 ml. of water in a Waring Blendor for eight two minute periods. The Blendor jar was kept cool between blending periods in an ice-bath. One ml. of 1:1000 aqueous merthiolate was added to inhibit bacterial growth. The suspension was then adjusted to a desired pH with dilute hydrochloric acid, transferred to a 500 ml. volumetric flask, and brought to volume with water. The contents of the flask were transferred to a one-pint Mason jar which was then placed in a water-bath maintained at a desired temperature. The muscle suspension was agitated throughout the course of each experiment by means of a mechanical stirrer. The pH ($\pm$ 0.05 units) was maintained by the addition of dilute hydrochloric acid or sodium hydroxide. The amount of acid or alkali added, usually less than one ml., was considered not to

change appreciably the volume of the suspension.

3. Preparation of deproteinized filtrates

At intervals during the incubation, two 25 ml. aliquots were withdrawn from the autolyzing suspension. One of these was deproteinized with trichloroacetic acid, the other with tungstic acid. To prepare the trichloroacetic acid filtrate, an equal volume of 10 per cent trichloroacetic acid was added to one of the 25 ml. aliquots from the suspension (5 per cent trichloroacetic acid final concentration). Three ml. each of 10 per cent sodium tungstate and 2/3 N sulfuric acid were added to the other aliquot for the tungstic acid filtrate. After thorough shaking, the precipitating mixtures were allowed to stand thirty minutes, and after centrifuging at 2000 rpm for five minutes filtered through No. 1 Whatman filter paper. The first portion of each filtrate was discarded as the paper retained some of the soluble nitrogen.

4. Nitrogen determination

Two types of nitrogen determinations were made; total-nitrogen and non-protein-nitrogen. To analyze for total-nitrogen, 5 ml. aliquots of the incubation suspension, in triplicate, were transferred to micro-Kjeldahl flasks, and

and 2 ml. of nitrogen-low sulfuric acid were added. The digestion was carried out in the usual fashion, using as catalyst a small amount of a 1:1 mixture of copper sulfate and potassium sulfate. When the digest was free of charred material, 30 per cent hydrogen peroxide was introduced, a few drops at a time, up to a maximum of ten drops. With further boiling the digest became clear and was used, as such, for the analysis. The entire digest was distilled into 4 per cent boric acid and titrated with 0.10 N hydrochloric acid according to the usual micro-Kjeldahl procedure. This permitted the calculation of the total-nitrogen in the original suspension.

Triplicate 2 ml. aliquots of each filtrate were used for the analysis of soluble-nitrogen (non-protein-nitrogen). The digestion and distillation were carried out as described for total nitrogen except that 0.01 N hydrochloric acid was used for titrating. This permitted the calculation of non-protein nitrogen in the suspension.

5. Effect of pH on autolysis

It is desirable to study an enzyme system at that pH and temperature which are optimum for its activity. Because these optima have not been defined for proteolysis in whole muscle suspensions, experimental work was undertaken to

define the optimum pH.

Suspensions of chicken breast muscle were incubated at several pH levels, all at 37° C. The optimum pH for maximum proteolytic activity at this temperature was established by noting the increase in trichloroacetic acid soluble-nitrogen in filtrates prepared from suspensions incubated at pH levels ranging from 6.00 to 3.50 (Table 1). Only the non-protein-nitrogen in trichloroacetic acid filtrates was measured.

The data establish a pH value of 4.00 as the optimum for proteolytic activity at 37° C. Since many workers have reported that cathepsin shows its greatest activity at approximately pH 4.00 and 37° C., it is assumed that it is this complex which is responsible for the decomposition of the tissue proteins observed here. Because tungstic acid filtrates were not prepared, it was not possible to follow the course of the proteolysis.

6. Effect of temperature on autolysis

The pH optimum having been established, a series of incubations were carried out to define the optimum temperature for proteolytic activity. The temperatures used ranged from 25° C. to 55° C. Trichloroacetic acid and tungstic acid filtrates of aliquots from the autolyzing suspensions were prepared and analyzed for non-protein nitrogen. The suspensions

Table 1

The Effect of pH on Rate of Autolysis of Minced Chicken Breast Muscle*

pH	No. of Replicates	Incubation Time Hours	Non-Protein-Nitrogen as Per Cent of Total Nitrogen
6.00	2	0	18.03
		12	18.54
		24	19.28
5.00	2	0	18.28
		12	18.66
		24	19.90
4.20	3	0	16.75
		12	17.92
		24	18.96
4.00	3	0	17.63
		12	23.13
		24	30.38
3.80	3	0	16.84
		12	17.98
		24	18.49
3.50	1	0	19.57
		12	19.57
		24	19.57

*Samples incubated at 37° C., protein-nitrogen precipitated by trichloroacetic acid.

were allowed to autolyze for only six hours because it had been determined that measurable changes occurred in that time interval. The results are shown in Table 2.

Table 2

Effect of Time and Temperature on Rate of Autolysis of Minced Chicken Breast Muscle*

Incubation Time - Hrs.		0		2		4		6	
Incubation Temp.	Reps.	TCA**	TA***	TCA	TA	TCA	TA	TCA	TA
25°C.	a	15.28	-	15.28	-	15.79	-	16.29	-
	b	-	-	-	-	-	-	-	-
35°C.	a	14.40	12.63	14.92	13.13	15.29	13.38	16.73	14.13
	b	13.43	-	14.65	-	14.92	-	15.84	-
40°C.	a	14.68	13.17	15.70	13.93	16.20	14.69	17.21	15.44
	b	14.88	13.97	15.42	14.76	15.93	15.93	17.23	16.47
45°C.	a	15.30	14.02	16.20	14.91	17.23	15.55	17.74	15.82
	b	15.90	13.11	16.67	13.74	17.05	13.99	17.72	15.78
55°C.	a	14.94	12.82	15.59	13.69	16.08	13.95	16.59	14.32
	b	15.94	14.13	16.98	15.68	17.72	15.68	18.00	15.95

* Samples incubated at pH 4.00.
** Non-protein-N in trichloroacetic acid filtrates as per cent of total-N.
*** Non-protein-N in tungstic acid filtrates as per cent of total-N.

It is evident from these data that the maximum rate of autolysis (proteolytic activity) occurs at 40° C. when tissue suspensions are incubated at pH 4.00. It is also evident that there is a greater degradation of protein to intermediate products rather than to peptides and amino acids. This is indicated by the greater amount and rate of formation of nitrogen in trichloroacetic acid filtrates and tungstic acid filtrates. The values for these nitrogen fractions become more divergent as time progresses. It appears that the decomposition of protein in chicken breast muscle by naturally occurring proteolytic enzymes produces molecules of intermediate size which, in turn, are broken down into still smaller fragments or molecules. If the protein molecule had been ruptured into a small fragment and a residue, the latter resembling the intact protein in its reaction to the precipitating agents used, then a greater increase in non-protein-nitrogen would have been observed in the tungstic acid filtrate.

7. Autolysis of beef muscle at pH 4.00 and 40° C.

Two experiments were carried out to determine the extent of autolysis in muscle from freshly-killed beef under the same conditions as those used in the studies on chicken breast muscle. The samples, from Canner-Cutter beef,

obtained from a commercial packer, were taken from the flank muscle. They were removed from the carcass thirty minutes after slaughter. After cutting away the visible fat and connective tissue, the samples were ground and thoroughly mixed. Duplicate 25 gram portions were used in each experiment; they were autolyzed at pH 4.00 and 40° C. Table 3 summarizes the results.

Table 3

Autolysis of Beef Flank Muscle
at pH 4.00 and 40°C.

Expt. No.	Incubation Time, Hours	Non-Protein Nitrogen in: Trichloroacetic Acid Filtrate % of Total Nitrogen	Tungstic Acid Filtrate % of Total Nitrogen
1a & b	0	11.55	11.13
	2	13.33	11.68
	4	14.79	11.91
2a & b	0	11.00	9.59
	2	12.10	10.40
	3½	12.93	10.74

These data show that the proteolysis in beef muscle suspensions, under these experimental conditions is quite similar to that observed for chicken breast muscle. As before, there was a greater formation of intermediate than end products of protein degradation. An interesting

observation is noted when the data from the two tissue incubations are compared (Tables 2 and 3). The change (increase in trichloroacetic-acid-soluble-nitrogen) is approximately the same in both cases. However, the rate of proteolysis is more rapid in beef since only four hours were necessary to bring about the same degree of protein decomposition that occurred in the chicken muscle in six hours. This finding substantiates that of Chen and Bradley (64). As previously mentioned, they observed that pigmented tissue autolyzed to a greater extent than non-pigmented tissue. Also beef muscle autolyzed to a greater extent than either the white or red muscle of chicken.

If the assumption is made that tenderization during storage is the result of autolysis, then the results of these experiments suggest that beef should tenderize faster than chicken meat. Actually such is not the case; beef usually requires a storage period of seven to fourteen days to tenderize, while chicken meat requires no more than twenty-four to forty-eight hours. However, it must be borne in mind that the conditions of these experiments differ from those existing during normal aging. While the difference in pH and temperature should have no effect on the rate of autolysis, except for an accelerating action, the condition of the muscle may have some effect. In a muscle suspension

the substrate may be more accessible to the proteolytic enzymes than it is in the intact muscle. In order to account for these results then, it must be assumed that the substrate is more accessible in suspensions of beef muscle than of chicken muscle, or that there is a greater concentration of these enzymes in beef tissue. The former is not probable, since it seems reasonable to believe that no difference in accessibility exists. The question of possible differences in enzyme concentration cannot be answered, since no data are available relative to the concentration of proteolytic enzymes in either beef or chicken muscle. Tentatively it is suggested that tenderization may not be entirely the result of autolysis. However, it must be emphasized that minute changes (often undetectable by analytical methods) in the chemical constitution of proteins produce extensive changes in the physical state. This has been demonstrated many times, both in classical and modern protein chemistry.

8. Comparative autolysis of beef and chicken meat

In light of the previous findings, it was decided to repeat the experiment at a pH more nearly like that of stored meat. Autolysis at this pH should more closely resemble that which occurs in meat. A value of 5.50 was selected. Because

enzymic activity at this pH would necessarily be slower, the suspensions were allowed to autolyze for 144 hours to insure recording measurable changes. The data presented in Table 4 show the comparison between the two types of tissue.

Table 4

Autolysis in Beef Flank and Chicken Breast Muscle at pH 5.50 and 40° C.

Muscle	Incubation Time, Hours	Non-Protein Nitrogen in: Trichloroacetic Acid Filtrate % of Total Nitrogen	Tungstic Acid Filtrate % of Total Nitrogen
Chicken	0	13.57	13.43
	24	16.40	14.30
	96	16.89	14.51
	144	17.21	14.79
Beef	0	11.43	10.05
	6	12.04	10.21
	24	12.37	10.60
	72	13.89	11.40
	144	15.84	12.88

It is evident that the extent of proteolysis during these trials is similar to that observed in other studies. It is also evident that the rate of autolysis of beef is greater than that for chicken meat, thus substantiating the findings at pH 4.00 and 40° C. Compare the increases in the two nitrogen fractions of both muscles. The increase in

trichloroacetic-acid-soluble-nitrogen was 38.76 per cent for beef and 26.82 per cent for chicken. The respective increases in tungstic-acid-soluble-nitrogen were 28.82 per cent and 10.13 per cent. It would appear then, considering the nature of the experimentation, that tenderization of meat during storage is the result of changes other than the enzymic decomposition of muscle proteins. The primary change during storage may involve an alteration in the physical state of the proteins. This alteration in the physical state could be the result of minute chemical changes in the proteins due to autolysis. However, the proteolysis observed in beef may be, at least in part, responsible for the "mushy" texture of canned beef.

B. Investigations on Canned Beef

The results of autolysis experiments suggests that proteolysis in meat during the initial stages of thermal processing may be responsible, in part, for the "mushy" texture encountered in canned beef. The thermal decomposition of protein may be another contributing factor. Thermal processing studies therefore were made to determine and characterize the chemical alterations occurring in meat during canning.

1. Design of the investigation

After much consideration, it was decided to study the effect of three processing variables at three levels on chemical changes in meat during processing. The variables selected were; size of the piece of meat, processing time, and retort (processing) temperature. Use was made of a 3 X 3 X 3 factorial experiment with three replications; treatments were assigned at random.

a. Size of the piece of meat

The size of the piece of meat was controlled by using three different tinplate containers (300 X 200, 300 X 308, and 401 X 411). The dimensions of these containers are 3" X 2", 3" X 3½", and 4 1/16" X 4 11/16", respectively. These containers were standard cans, the interior surface of which was coated with lacquer while the exterior was over-coated with an olive drab finish used on Army canned items. The can lids were of the conventional type, finished in a manner similar to the cans, and possessed a rubber sealing compound within the curl around the circumference of the lid. All cans were sealed with an automatic closing machine operating under a vacuum of 29 inches and forming a double seam on the can. One solid piece of meat, six ounces for the 300 X 200 can, twelve ounces for the 300 X 308 can, and

thirty ounces for the 401 X 411 can, cut from the muscle was used; it completely filled the can.

b. The selection of grade and cut of beef

Commercial practice was followed in selecting beef grading Canner-Cutter according to United States Department of Agriculture standards. Because a solid piece necessary to fill the 401 X 411 can had to weigh thirty ounces and measure 4 1/16 inches by 4 11/16 inches, the anterior portion of the biceps femoris of outside rounds weighing twelve to fourteen pounds was used. The outside round is the only cut used in commercial practice from which such a piece could be obtained. Since it was not possible to obtain the samples for the three different can sizes from the same round, one round was used for each can.

c. The retort (processing) temperatures

The processing temperatures selected were 220° F. (104.4° C.), 235° F. (112.8° C.), and 250° F. (121.1° C.). These were chosen to determine the influence of processing temperature on the rate and extent of the chemical changes which may occur during processing.

d. Calculation of processing times

Processing times were calculated according to the Graphical Method of Bigelow (75) from heat penetration studies made on cans of beef packed as described and the thermal death time curve for Putrefactive Anaerobe 3679 having a $z = 16$. The z value is the slope of the thermal death time curve. It is the number of degrees Fahrenheit encompassed by the thermal death time curve in passing through one log cycle on the time scale.

For the heat penetration studies, six cans of each size were packed (as described) with beef from which all visible fat and connective tissue had been removed. The cans, six of each size, were processed at the three temperatures in a vertical type, laboratory size retort. The cans were so placed that the movement of steam within the retort was parallel to the fibers of the meat in the can. During the processing the temperature at the center of each can was measured with the appropriate size Ecklund molded plastic, copper-constantan thermocouple and two Brown Electronik, twelve point, strip-chart, self-balancing potentiometers. The temperature of processing was maintained at the desired level by use of a Taylor temperature controller. The data for the six cans of each size were averaged to obtain the rate of heat penetration.

The thermal death time curve for Putrefactive Anaerobe 3679 was selected because this organism is one of the most heat resistant anaerobes known. A z value of 16 was chosen after careful consideration of the work of Gross et al. (76) since they have shown that this value is more nearly correct for meat than the usually assumed value of 18. The thermal death time curves were given F values of 3.5, 5, and 7. F is the number of minutes required to destroy an organism at 250° F. In order to calculate three processing times for each can size at each processing temperature, it was necessary to have three F values. Gross (77) has shown that for a load of 10,000 spores of Putrefactive Anaerobe 3679 per gram of meat, an F value of 3.5 is necessary to insure commercial sterility. However, this load of organisms is much less than commonly recognized commercially, although work at Iowa State College (78) is demonstrating that even 10,000 spores per gram is in excess of the actual load. Commercial packers process for a much higher initial contamination, using F_o values up to as high as 20. Since this study was a combination of the academic and commercial approach a value of 3.5 was selected. The values of 5 and 7 were used to give processing time to sterilize for loads approaching those believed existing by industry. The calculated processing times (in minutes) are presented in Table 5.

Table 5

Calculated Processing Times

Can Size	F Value	Retort Temperatures 220° F. Minutes	235° F. Minutes	250° F. Minutes
300 X 200	3.5	247	72	47
	5.0	471	89	50
	7.0	641	124	53
300 X 308	3.5	370	101	67
	5.0	497	118	70
	7.0	676	147	73
401 X 411	3.5	414	163	112
	5.0	589	178	116
	7.0	724	199	123

_e_. _Sampling of the meat_

It has been stated that one outside round was used as the sample for each can. Since there were twenty-seven processes, each in triplicate, eighty-one rounds were required. To insure random distribution in sampling, the 81 rounds were obtained from a commercial packer in three lots of 27 rounds each. Each lot was received at weekly intervals. The rounds were removed from the shipping container and assigned at random a number from 1 to 27. Then these 27 rounds were randomly subdivided into nine groups of three rounds each. The three rounds were used for the replications at some retort temperature and some processing time.

f. Statistical design

The complete design is shown in Table 6.

Table 6

Statistical Design for Processing Beef

Block	Processing Temperature	Can Size 300 X 200 F	Min.	300 X 308 F	Min.	401 X 411 F	Min.
I	220° F.	3.5	(274)	5.0	(497)	7.0	(724)
	235° F.	5.0	(89)	7.0	(147)	3.5	(163)
	250° F.	7.0	(53)	3.5	(67)	5.0	(116)
II	220° F.	5.0	(471)	7.0	(676)	3.5	(414)
	235° F.	7.0	(124)	3.5	(101)	5.0	(178)
	250° F.	3.5	(47)	5.0	(70)	7.0	(123)
III	220° F.	7.0	(641)	3.5	(370)	5.0	(589)
	235° F.	3.5	(73)	5.0	(118)	7.0	(199)
	250° F.	5.0	(50)	7.0	(73)	3.5	(112)

The treatments within each block were processed at random.

2. Preparation of the raw beef for canning

The meat was taken from the refrigerator (40° F.), trimmed free of excess fat and connective tissue, and cut into pieces to fit the particular can size. The meat was placed in the can so that the fibers were parallel to the longer axis of the container. The cans were vacuum sealed

and placed in the 40° F. refrigerator for twelve to eighteen hours before processing was begun. At the same time as the cans were packed, portions of each round were placed in a can, sealed, and frozen at -30° F. They were held at this temperature pending analysis for control values.

3. Heat processing procedure

After placing the cans in the retort, the lid was sealed by tightening the lid clamps of the retort. Compressed air was run into the retort until the desired pressure level had been reached. At this point steam was introduced into the retort. The process time was started when the Taylor controller indicated that the retort had reached the processing temperature and continued until the steam was shut off and cooling was begun. Cooling was accomplished under pressure with cold water. The cans were held approximately thirty days before analysis.

4. Procedure for analyzing processed cans

After opening the can, the piece of meat was removed, placed on a sieve, and allowed to drain for three minutes. The juice was reserved for subsequent analysis. The weight of the piece of meat was determined to the nearest gram. This permitted the calculation of per cent loss in weight

on processing.

a. Determination of the pH of the processed meat

Portions of the meat and juice were recombined in the same ratio as found in the processed can. After thorough mixing (in a Waring Blendor), the pH was measured with a Model G Beckman pH meter. The pH of the raw meat had been recorded at the time the can was packed by inserting penetrating glass electrodes directly into the muscle.

b. Preparation of samples for analysis

Total nitrogen and non-protein nitrogen (the latter in trichloroacetic acid and tungstic filtrates) determinations were made on a mixture of the homogenized meat and juice and on the juice alone. Duplicate 5 per cent suspensions from 25 gram portions of the homogenate were prepared following the procedure described previously. One exception was made. Only one three-minute homogenizing period was used. Deproteinized filtrates were prepared in the same manner as previously described. The micro-Kjeldahl procedure was used of the determination of total-nitrogen and non-protein nitrogen.

For the juice "suspension", the same weight of juice as was used in the homogenate was brought to a volume of

500 ml. with water. The same nitrogen determinations were made. This permitted the calculation of the amount of the constituents in the juice as they were expressed from the meat during processing.

5. Results

The results are presented in tabular form in the following pages. The figures were obtained by averaging the results of three determinations.

a. Effect of processing on the nitrogen fractions in beef

The analytical data are presented in Tables 7 to 9 inclusive. It will be noted that the amount of nitrogen soluble in trichloroacetic acid increased during processing (indicating that a decomposition of protein material occurred). The average increases were 28.37 per cent, 31.08 per cent, and 24.99 per cent for can sizes 300 X 200, 300 X 308, and 401 X 411 respectively. The average increase for all can sizes was 28.15 per cent. The non-protein nitrogen in the tungstic filtrate increased, remained unchanged, or, in many instances, decreased. One reason for this decrease could have been the fact that more nitrogen-containing constituents of the processed meat were precipitated by the action of the tungstic acid. However, this is not probable since the lower

Table 7

Effect of Processing in Can Size 300 X 200 on Nitrogen Fractions in Beef

Processing		Non-Protein Nitrogen* in: Raw Meat		Processed Meat	
		TCA**	TA***	TCA	TA
Temp. °F.	Time Min.	% of Total-N	% of Total-N	% of Total-N	% of Total-N
220	247	13.46	11.63	18.78	10.90
		12.40	11.02	16.71	10.83
		12.72	10.66	15.88	11.14
	471	10.91	10.58	15.31	10.49
		13.58	10.97	16.19	11.23
		13.51	11.28	16.92	11.28
	641	10.86	10.25	17.98	11.28
		11.26	10.13	18.09	10.65
		10.67	11.02	18.90	12.16
235	72	12.35	9.21	13.58	10.45
		11.84	8.88	12.79	9.92
		11.73	10.31	13.60	9.96
	89	11.67	10.79	14.56	10.26
		13.68	11.11	16.42	11.40
		13.41	11.76	14.60	10.19
	124	13.45	11.69	15.10	11.60
		10.53	9.95	15.67	9.70
		13.13	12.30	17.60	11.82
250	47	12.66	10.55	12.74	8.86
		14.08	12.06	14.36	11.05
		13.52	10.58	12.85	9.57
	50	10.26	8.89	13.14	10.18
		10.48	8.83	13.48	10.25
		9.16	8.61	12.80	9.40
	53	11.78	10.10	13.70	10.34
		13.09	10.88	13.78	10.46
		13.16	11.05	14.21	9.47

* Values are average of three determinations.
** Trichloroacetic Acid Filtrate.
*** Tungstic Acid Filtrate.

Table 8

Effect of Processing in Can Size 300 X 308 on Nitrogen Fractions in Beef

Processing		Non-Protein Nitrogen* in:			
		Raw Meat		Processed Meat	
Temp. °F.	Time Min.	TCA** % of Total-N	TA*** % of Total-N	TCA % of Total-N	TA % of Total-N
220	370	11.33	9.56	20.61	10.21
		11.43	9.35	16.10	10.39
		12.21	9.83	15.15	10.47
	497	12.13	10.32	15.35	9.90
		12.87	11.26	15.55	10.37
		13.41	11.38	16.44	11.48
	676	12.11	9.85	18.70	11.38
		12.30	10.23	17.75	12.96
		10.63	9.74	16.62	11.80
235	101	10.14	8.70	13.81	9.97
		9.69	8.70	13.05	9.85
		10.53	9.35	13.03	10.03
	118	10.01	10.20	15.30	9.35
		12.65	10.39	14.12	9.71
		12.97	9.80	14.99	9.51
	147	12.10	10.41	14.97	8.88
		11.48	10.13	13.92	8.94
		11.77	10.57	15.21	9.28
250	67	12.14	10.94	13.68	10.00
		12.74	10.96	15.78	9.89
		11.95	9.43	13.41	9.27
	70	9.96	9.06	13.09	8.89
		10.67	9.07	14.40	9.07
		10.56	9.81	14.44	9.72
	73	10.83	9.26	14.44	10.00
		10.16	9.63	13.47	8.82
		11.08	8.99	15.26	9.08

* Values are average of three determinations.
** Trichloroacetic Acid Filtrate.
*** Tungstic Acid Filtrate

Table 9

Effect of Processing in Can Size 401 X 411 on Nitrogen Fractions in Beef

Processing		Non-Protein Nitrogen* in: Raw Meat		Processed Meat	
		TCA**	TA***	TCA	TA
Temp. °F.	Time Min.	% of Total-N	% of Total-N	% of Total-N	% of Total-N
220	414	12.04	12.13	15.97	10.21
		14.45	12.23	14.89	10.64
		12.92	11.67	13.90	9.36
	589	12.03	10.23	17.90	10.23
		12.11	10.43	16.27	9.81
		10.35	9.46	14.43	9.62
	724	13.24	11.39	19.44	11.39
		13.02	11.11	15.03	9.75
		13.28	11.11	16.26	10.30
235	163	13.17	10.44	16.89	10.90
		14.22	11.82	15.38	10.93
		13.05	11.36	14.48	9.65
	178	11.54	10.26	15.35	9.81
		12.23	11.44	14.36	9.84
		10.08	8.72	13.76	9.40
	199	11.14	9.12	16.23	11.23
		11.99	10.58	13.49	9.26
		13.06	11.39	14.17	9.72
250	112	10.28	9.92	16.80	11.38
		12.57	9.98	16.31	10.70
		12.69	9.86	16.35	10.50
	116	13.19	11.81	17.67	10.71
		14.57	13.07	18.56	11.48
		12.76	10.05	14.67	9.09
	123	13.81	12.03	15.60	11.76
		13.72	11.23	15.87	10.70
		13.56	12.53	16.34	11.05

* Values are average of three determinations.
** Trichloroacetic Acid Filtrate.
*** Tungstic Acid Filtrate.

values appear to be distributed irregularly. Okuda (72) reported the formation of ammonia in meat during processing. Moreover, more ammonia was formed in the samples with the higher pH values. A loss of ammonia could account for the lower values in some of the processed meat. Here also the formation of ammonia (if the loss is a result of this) seems to be correlated with the pH value of the raw meat. Whenever there is a considerable difference in the tungstic-acid-filtrate-nitrogen between the raw and processed meat, the pH value of the sample (measured on the raw meat) was 6.00 or higher. In those instances where the difference was not as large, the pH of the raw meat was not much below this value.

It is interesting to compare the changes in the trichloroacetic-acid-filtrate-nitrogen in beef due to the effects of processing with those in the same fraction due to autolysis. An increase (in both cases) indicates a degradation of proteins. A difference between trichloroacetic-acid-filtrate-nitrogen and tungstic-acid-filtrate-nitrogen (also in both cases) indicates the formation of decomposition products of intermediate size. This is more obvious for the processed beef, if it is considered that no change occurred in the tungstic-acid-filtrate-nitrogen (the average value for this fraction in the raw meat for all can

sizes was the same as the average value in the processed meat).

The statistical analyses of these data are presented in Tables 10 to 15. A preliminary analysis of variance indicated that a triple interaction existed with respect to many of the variables (the various nitrogen fractions) involved as between processing time, processing temperature, and can size. Therefore, the procedure adopted was that of analyzing the data for each can size separately, i.e., this meant handling the data for each can size as a separate experiment. Regression analysis was used to obtain the relationship of each of the variables with time since processing times differed with the three F values within each temperature. To secure the effect of temperature at an average time (since this effect could not be obtained directly from the data) the method of adjustment to an average time by means of the regression coefficient was used, and the adjusted means were tested for significance.

The assumption was made that the relationship of the variables measured is linear relative to time. (It is quite evident that this assumption is probably not correct; however, any other assumption seemed impractical since only three processing times were used within each temperature.) The serious difficulty with this assumption is the possibility

Table 10

Regression and Correlation Data for Effect of Processing on Trichloroacetic-Acid-Filtrate-Nitrogen Fraction in Beef

Group	Degrees of Freedom	Correlation Coefficient	Regression Coefficient[1]	Errors of Estimate Sum of Squares	Errors of Estimate d.f.
1	8	0.749	0.00089	0.1443	7
2	8	0.660	0.0039	0.0844	7
3	8	0.300	0.177	0.0208	7
Sub-Total	24	0.564**	0.00094	0.4555	23
4	8	0.123	0.00019	0.3291	7
5	8	-0.226	-0.00139	0.1171	7
6	8	0.803**	0.03048	0.0278	7
Sub-Total	24	0.085	0.00016	0.5316	23
7	8	0.403	0.000497	0.1849	7
8	8	0.167	0.00146	0.1496	7
9	8	-0.664	-0.2105	0.1072	7
Sub-Total	24	0.246	0.00048	0.5298	23
Total	72	0.330**	0.000603	1.5748	71

[1] All regression coefficients are significantly greater than zero.
** Significant at P = .01.

Table 11

Adjusted Means for Effect of Processing on Trichloroacetic-Acid-Filtrate-Nitrogen Fraction in Beef

Can Size	Processing Temp. °F.	Mean Change	Adjusted Mean
300 X 200	220	1.43	1.19
	235	1.20	1.30
	250	1.14	1.27
300 X 308	220	1.14	1.37
	235	1.28	1.30
	250	1.28	1.31
401 X 411	220	1.28	1.14
	235	1.22	1.28
	250	1.28	1.36

Table 12

Analysis of Covariance and Test of Significance of Adjusted Means of Effect of Processing on Trichloroacetic-Acid-Filtrate-Nitrogen Fraction in Beef

Source of Variation	Degrees of Freedom	Errors of Estimate: Sum of Squares	Errors of Estimate: Degrees of Freedom	Errors of Estimate: Mean Square	F Test
Between temperatures in can size 300 X 200					
Total	25	0.4728	24		
Treatments	2				
Error (within groups)	23	0.4555	22	0.0198	
For test of significance of means		0.0173	2	0.00865	0.437
Between temperatures in can size 300 X 308					
Total	25	0.6314	24		
Treatments	2				
Error (within groups)	23	0.5316	22	0.0231	
For test of significance of means		0.0998	2	0.0499	2.158
Between Temperatures in can size 401 X 411					
Total	25	0.5700	24		
Treatments	2				
Error (within groups)	23	0.5298	22	0.0230	
For test of significance of means		0.0402	2	0.0201	0.874

Table 13

Regression and Correlation Data for Effect of Processing on Tungstic-Acid-Filtrate-Nitrogen Fraction in Beef

Group	Degrees of Freedom	Correlation Coefficient	Regression Coefficient[1]	Errors of Estimate Sum of Squares	d.f.
1	8	0.710	0.00022	0.0109	7
2	8	0.515	0.00190	0.0419	7
3	8	0.213	0.01000	0.1133	7
Sub-Total	24	0.276	0.0025	0.1829	23
4	8	-0.130	-0.000113	0.1036	7
5	8	-0.899**	-0.005046	0.0941	7
6	8	0.553	0.011667	0.0167	7
Sub-Total	24	-0.173	-0.00022	0.2249	23
7	8	0.604	0.000348	0.0326	7
8	8	0.164	0.001361	0.0995	7
9	8	0.601	0.012258	0.0495	7
Total	72	0.138	0.000158	0.6780	71

[1] All regression coefficients are significantly greater than zero.

** Significant at P = .01.

Table 14

Adjusted Means for Effect of Processing on Tungstic-Acid-Filtrate-Nitrogen Fraction in Beef

Can Size	Processing Temperature °F.	Mean Change	Adjusted Mean
300 X 200	220	1.02	0.96
	235	1.00	1.02
	250	0.99	1.03
300 X 308	220	1.09	1.15
	235	0.98	0.95
	250	0.97	0.93
401 X 411	220	0.92	0.81
	235	0.96	1.00
	250	0.98	1.05

Table 15

Analysis of Covariance and Test of Significance of Adjusted Means for Effect of Processing on Tungstic-Acid-Filtrate-Nitrogen Fraction in Beef

Source of Variation	Degrees of Freedom	Errors of Estimate: Sum of Squares	Errors of Estimate: Degrees of Freedom	Errors of Estimate: Mean Square	F Test
Between temperatures in can size 300 X 200					
Total	25	0.1888	24		
Treatments	2				
Error (within groups)	23	0.1829	22	0.0080	
For test of significance of means		0.0059	2	0.0030	0.385
Between temperatures in can size 300 X 308					
Total	25	0.2887	24		
Treatments	2				
Error (within groups)	23	0.2249	22	0.0104	
For test of significance of means		0.0638	2	0.0319	0.107
Between temperatures in can size 401 X 411					
Total	25	0.2464	24		
Treatments	2				
Error (within groups)	23	0.2249	22	0.0098	
For test of significance of means		0.0215	2	0.0108	1.102

that both a rising and falling trend with processing time may exist within the total range of each time used. A test of this hypothesis would require the use of several additional processing times within each temperature and can size. Also, the use of the adjusted means in determining the differences between temperatures has an implied assumption of a linear relationship with temperature. However, this does not appear to be in serious question since the range of temperatures used is comparatively small.

Many of the analyses used have involved percentage changes, or have included one variable expressed as a proportion of another. A preliminary check of the constancy of the error of variances was made and an almost undiscernible dependence of variance upon size of lot mean could be seen. It is recognized that such a dependence may exist and conclusions from such analyses modified accordingly.

In the analyses, use is made of the terms, regression coefficient, correlation coefficient, and adjusted mean. The regression coefficient (symbolized by b) is a rate defined by the expression $\Sigma XY/\Sigma X^2$, i.e., the rate of change in the Y variate with change in the X variate. It is the change in a nitrogen fraction (in a particular can size, group of can sizes, or all can sizes) with unit time

(minutes). The correlation coefficient (symbolized by r) denotes the relationship between the two variates. The adjusted mean shows the effect of processing temperature when the effect of varying time is removed. The "F" test was used to test for differences between the means due to processing temperatures. The value of "F" was determined by dividing the mean square for the treatments term by the mean square of the error term. If the "F" value so obtained was greater than that of a tabular "F" possessing the same number of degrees of freedom, the differences between adjusted means were said to be significant.

In the tables showing correlation and regression data, the heading "Group" refers to a can size which has been processed for three lengths of time at one of the processing temperatures. Thus, Groups 1, 2, and 3 designate processing in can size 300 X 200 at 220° F., 235° F., and 250° F. respectively, Groups 4, 5, and 6 in can size 300 X 308, and Groups 7, 8, and 9 in can size 401 X 411 all at the three temperatures. The sub-totals represent the summation for each can size; the total represents the summation for all can sizes.

The relationship of the change in trichloroacetic-acid-filtrate-nitrogen (expressed as the ratio of the processed meat value to the raw meat value) with processing time for

all can sizes appeared to be positive (b = 0.000603), although the relationship differed considerably between can sizes (Table 10). No clear relationship was evident with processing temperature (adjusted means) except within can size 401 X 411, where the relationship appeared to be positive. However, in no case was there a significant difference between adjusted means.

The change in tungstic-acid-filtrate-nitrogen appeared to be positively related to processing time (b = 0.000158) for all can sizes (Table 13). A positive relationship was also evident with processing temperature in can sizes 300 X 200 and 401 X 411, and a negative relationship with temperature in can size 300 X 308.

b. Effect of processing on the nitrogen fractions in juice expressed from beef

The data obtained from the fractionation of the juice expressed from the beef during processing are presented in Tables 16 to 18 inclusive. The juice was fractionated into total-nitrogen, protein-nitrogen, trichloroacetic-acid-filtrate-nitrogen, and tungstic-acid-filtrate-nitrogen. The value for protein-nitrogen was obtained by subtracting the trichloroacetic-acid-filtrate-nitrogen (non-protein-nitrogen) from the total-nitrogen. This procedure was considered permissible since Hiller and van Slyke (74) had

Table 16

Effect of Processing in Can Size 401 X 411 on Nitrogen Fraction in Juice Expressed from Beef During Processing

Processing Temp. °F.	Processing Time Min.	Total-N Gm. %	Protein-N Gm. %	Non-Protein Nitrogen* in: Trichloroacetic Acid Filtrate Gm. %	Non-Protein Nitrogen* in: Tungstic Acid Filtrate Gm. %
220	414	0.36	0.00	0.36	0.21
		0.43	0.18	0.25	0.14
		0.36	0.06	0.39	0.18
	589	0.45	0.06	0.39	0.18
		0.42	0.08	0.34	0.17
		0.37	0.09	0.28	0.16
	724	0.52	0.07	0.45	0.22
		0.37	0.08	0.29	0.15
		0.39	0.08	0.31	0.17
235	163	0.36	0.04	0.32	0.19
		0.41	0.10	0.31	0.15
		0.36	0.04	0.32	0.16
	178	0.35	0.03	0.32	0.16
		0.35	0.04	0.31	0.19
		0.31	0.03	0.28	0.17
	199	0.38	0.03	0.35	0.21
		0.35	0.10	0.25	0.14
		0.38	0.12	0.26	0.15
250	112	0.41	0.08	0.33	0.19
		0.38	0.05	0.33	0.20
		0.37	0.04	0.33	0.19
	116	0.38	0.00	0.38	0.20
		0.41	0.01	0.40	0.21
		0.37	0.05	0.32	0.21
	123	0.37	0.05	0.32	0.21
		0.38	0.03	0.35	0.21
		0.39	0.02	0.37	0.22

* Values are average of three determinations.

Table 17

Effect of Processing in Can Size 300 X 200 on Nitrogen Fractions in Juice Expressed from Beef During Processing

Processing Temp. °F.	Processing Time Min.	Total-N Gm. %	Protein-N Gm. %	Non-Protein Nitrogen* in: Trichloroacetic Acid Filtrate Gm. %	Non-Protein Nitrogen* in: Tungstic Acid Filtrate Gm. %
220	247	0.26	0.00	0.26	0.18
		0.30	0.02	0.28	0.20
		0.28	0.00	0.28	0.19
	471	0.44	0.06	0.38	0.22
		0.43	0.04	0.39	0.22
		0.42	0.06	0.36	0.23
	641	0.60	0.19	0.41	0.21
		0.53	0.08	0.45	0.21
		0.51	0.09	0.42	0.22
235	72	0.27	0.02	0.25	0.19
		0.25	0.00	0.25	0.20
		0.30	0.06	0.24	0.19
	89	0.30	0.02	0.28	0.20
		0.30	0.01	0.29	0.21
		0.30	0.01	0.29	0.21
	124	0.38	0.08	0.30	0.21
		0.31	0.00	0.31	0.20
		0.36	0.06	0.30	0.21
250	47	0.28	0.02	0.26	0.19
		0.29	0.03	0.26	0.21
		0.29	0.03	0.26	0.21
	50	0.30	0.00	0.30	0.22
		0.19	0.00	0.19	0.14
		0.30	0.00	0.30	0.22
	53	0.33	0.05	0.28	0.23
		0.32	0.04	0.28	0.21
		0.29	0.02	0.27	0.19

* Values are average of three determinations.

Table 18

Effect of Processing in Can Size 300 X 308 on Nitrogen Fractions in Juice Expressed from Beef During Processing

Processing Temp. °F.	Processing Time Min.	Total-N Gm. %	Protein-N Gm. %	Non-Protein Nitrogen* in: Trichloroacetic Acid Filtrate Gm. %	Non-Protein Nitrogen* in: Tungstic Acid Filtrate Gm. %
220	370	0.40	0.01	0.39	0.16
		0.30	0.02	0.28	0.19
		0.35	0.04	0.31	0.20
	497	0.40	0.01	0.39	0.22
		0.40	0.07	0.33	0.22
		0.39	0.06	0.33	0.22
	676	0.47	0.05	0.42	0.21
		0.50	0.15	0.35	0.23
		0.51	0.09	0.41	0.24
235	101	0.32	0.02	0.30	0.21
		0.31	0.02	0.29	0.22
		0.31	0.02	0.29	0.22
	118	0.31	0.06	0.24	0.17
		0.30	0.06	0.24	0.17
		0.30	0.04	0.26	0.15
	147	0.33	0.02	0.31	0.16
		0.32	0.04	0.28	0.17
		0.33	0.02	0.31	0.15
250	67	0.33	0.02	0.31	0.23
		0.35	0.02	0.33	0.20
		0.35	0.03	0.30	0.21
	70	0.30	0.01	0.29	0.19
		0.32	0.04	0.28	0.17
		0.33	0.04	0.29	0.18
	73	0.30	0.02	0.28	0.19
		0.32	0.05	0.27	0.17
		0.32	0.05	0.27	0.15

* Values are average of three determinations.

demonstrated that trichloroacetic acid in a final concentration of 5 per cent or less precipitated only protein from solution.

It is apparent that the amount of total-nitrogen in the juice expressed from the beef during processing (in the first two can sizes) is dependent upon the processing time at a particular processing temperature. This indicates the formation of decomposition products, and the relationship of their formation with time. An increase with time is also observed in the values for protein nitrogen. The non-protein-nitrogen in the trichloroacetic acid and tungstic acid filtrates seemed to vary too irregularly to indicate any trends. However, the difference in the amount of nitrogen between these two fractions (as was observed in the autolysis studies) again indicates the formation of decomposition products, which in turn indicates an alteration in the physical condition of the meat. The next set of tables, 19 to 30, contain the data on the statistical analyses of these four fractions. For the purposes of the analyses, the total-nitrogen in the juice was expressed as a per cent of the total-nitrogen of the homogenate, the trichloroacetic-acid-filtrate-nitrogen was expressed as a per cent of the trichloroacetic-acid-filtrate-nitrogen of the homogenate, the tungstic-acid-filtrate-nitrogen was

Table 19

Regression and Correlation Data
for Total-Nitrogen in Juice
as Per Cent of Total-Nitrogen in Homogenate

Group	Degrees of Freedom	Correlation Coefficient	Regression Coefficient[1]	Errors of Estimate Sum of Squares	d.f.
1	8	0.980**	0.0159	2.4080	7
2	8	0.730*	0.0398	5.8541	7
3	8	0.252	0.1078	9.2597	7
Sub-Total	24	0.870	0.016332	20.3501	23
4	8	0.880**	0.0132	7.5393	7
5	8	0.332	0.0069	1.2363	7
6	8	-0.043	-0.0094	2.6539	7
Sub-Total	24	0.825**	0.013044	11.5835	23
7	8	0.380	0.0049	20.3663	7
8	8	-0.190	-0.0041	2.9964	7
9	8	-0.097	-0.0194	7.3539	7
Sub-Total	24	0.310	0.004723	31.0478	23
Total	72	0.727**	0.0124	72.8210	71

[1] All regression coefficients are significantly greater than zero.
* Significant at P = .05.
** Significant at P = .01.

Table 20

Adjusted Means for Total-Nitrogen in Juice as Per Cent Total-Nitrogen in Homogenate

Can Size	Processing Temperature °F.	Mean Per Cent	Adjusted Mean Per Cent
300 X 200	220	11.00	6.81
	235	8.27	9.99
	250	7.17	9.63
300 X 308	220	11.15	7.53
	235	8.30	9.77
	250	8.52	10.67
401 X 411	220	10.94	9.61
	235	9.78	10.29
	250	10.44	11.25

Table 21

Analysis of Covariance and Test of Significance of Adjusted Means for Total-Nitrogen in Juice as Per Cent Total-Nitrogen in Homogenate

Source of Variation	Degrees of Freedom	Sum of Squares	Errors of Estimate: Degrees of Freedom	Errors of Estimate: Mean Square	F Test
Between temperatures in can size 300 X 200					
Total	25	32.2689	24		
Treatments	2				
Error	23	20.3501	22	0.8848	
For test of significance of means		11.9188	2	5.9594	6.740**
Between temperatures in can size 300 X 308					
Total	25	18.5378	24		
Treatments	2				
Error	23	11.5835	22	0.5036	
For test of significance of means		6.9543	2	3.4772	6.910**
Between temperatures in can size 401 X 411					
Total	25	35.0437	24		
Treatments	2				
Error	23	31.0478	22	1.3499	
For test of significance of means		3.9959	2	1.9980	1.481

** Significant at P = .01.

Table 22

Regression and Correlation Data for Trichloroacetic-Acid-Filtrate-Nitrogen (Juice) as Per Cent of Trichloroacetic-Acid-Filtrate-Nitrogen (Homogenate)

Group	Degrees of Freedom	Correlation Coefficient	Regression Coefficient[1]	Errors of Estimate Sum of Squares	d.f.
1	8	-0.023	-0.0012	634.6420	7
2	8	-0.056	-0.0068	60.3859	7
3	8	-0.043	-0.117	361.9192	7
Sub-Total	24	-0.020	-0.0014	1058.5106	23
4	8	0.649	0.0298	172.2134	7
5	8	-0.483	-0.0577	35.3673	7
6	8	-0.649	-0.07167	38.0242	7
Sub-Total	24	0.519**	0.0277	298.7813	23
7	8	0.037	0.0057	347.0336	7
8	8	-0.352	-0.863	102.7573	7
9	8	0.405	0.3332	105.4252	7
Sub-Total	24	0.077	0.0049	591.6418	23
Total	72	0.420**	0.0083	2027.8415	71

[1] All regression coefficients are significantly greater than zero.

** Significant at P = .01.

Table 23

Adjusted Means for
Trichloroacetic-Acid-Filtrate Nitrogen (Juice)
as Per Cent of
Trichloroacetic-Acid-Filtrate-Nitrogen (Homogenate)

Can Size	Processing Temperature °F.	Mean Per Cent	Adjusted Mean
300 X 200	220	54.93	55.29
	235	51.02	50.87
	250	49.92	49.71
300 X 308	220	56.84	49.11
	235	52.45	56.15
	250	54.15	58.73
401 X 411	220	55.23	53.83
	235	54.99	55.53
	250	57.68	58.53

Table 24

Analysis of Covariance and Test of Significance of Adjusted Means for Trichloroacetic-Acid-Filtrate-Nitrogen (Juice) as Per Cent Trichloroacetic-Acid-Filtrate-Nitrogen (Homogenate)

Source of Variation	Degrees of Freedom	Errors of Estimate Sum of Squares	Degrees of Freedom	Mean Square	F Test
Between temperatures in can size 300 X 200					
Total	25	2219.005	24		
Treatments	2				
Error	23	1058.5106	22	46.0222	
For test of significance of means		1160.4949	2	580.2475	12.608**
Between temperatures in can size 300 X 308					
Total	25	368.7246	24		
Treatments	2				
Error	23	298.7813	22	12.9905	
For test of significance of means		69.9433	2	34.9717	2.692
Between temperatures in can size 401 X 411					
Total	25	625.7419	24		
Treatments	2				
Error	23	591.6418	22	25.7236	
For test of significance of means		34.1001	2	17.0501	0.663

** Significant at $P = .01$.

Table 25

Regression and Correlation Data for Tungstic-Acid-Filtrate Nitrogen (Juice) as Per Cent of Tungstic-Acid-Filtrate-Nitrogen (Homogenate)

Group	Degrees of Freedom	Correlation Coefficient	Regression Coefficient[1]	Errors of Estimate Sum of Squares	d.f.
1	8	0.208	0.0034	60.7632	7
2	8	-0.398	-0.0353	27.8632	7
3	8	-0.092	-0.2528	407.9359	7
Sub-Total	24	0.058	0.0027	506.2881	23
4	8	0.331	0.0102	121.1537	7
5	8	-0.812**	-0.1989	66.4213	7
6	8	-0.721	-1.0294	52.8613	7
Sub-Total	24	0.094	0.0052	437.0199	23
7	8	0.058	0.0027	307.8235	7
8	8	0.006	0.0211	234.8297	7
9	8	0.298	0.2037	79.1947	7
Sub-Total	24	0.048	0.0032	629.9931	23
Total	72	0.642**	0.0035	1573.8800	71

[1] All regression coefficients are significantly greater than zero.

** Significant at $P = .01$.

Table 26

Adjusted Means for
Tungstic-Acid-Filtrate-Nitrogen (Juice)
as Per Cent Tungstic-Acid-Filtrate-Nitrogen (Homogenate)

The Adjusted Means			
Can Size	Processing Temperature °F.	Mean Per Cent	Adjusted Mean
300 X 200	220	49.74	49.06
	235	51.91	52.19
	250	51.21	51.61
300 X 308	220	51.30	49.85
	235	48.99	49.68
	250	52.47	53.33
401 X 411	220	46.05	45.14
	235	45.59	45.94
	250	51.49	52.05

Table 27

Analysis of Covariance and Test of Significance of Adjusted Means for Tungstic-Acid-Filtrate-Nitrogen (Juice) as Per Cent Tungstic-Acid-Filtrate-Nitrogen (Homogenate)

Source of Variation	Degrees of Freedom	Sum of Squares	Degrees of Freedom	Mean Square	F Test
Between temperatures in can size 300 X 200					
Total	25	519.8668	24		
Treatments	2				
Error	23	506.2881	22	22.0125	
For test of significance of means		13.5787	2	6.7894	0.308
Between temperatures in can size 300 X 308					
Total	25	495.9811	24		
Treatments	2				
Error	23	437.0199	22	19.0009	
For test of significance of means		58.9612	2	29.4806	1.552
Between temperatures in can size 401 X 411					
Total	25	779.5314	24		
Treatments	2				
Error	23	629.9930	22	27.3910	
For test of significance of means		149.5384	2	74.7692	2.730

Table 28

Regression and Correlation Data
for Protein-Nitrogen in the Juice

Group	Degrees of Freedom	Correlation Coefficient	Regression Coefficient[1]	Errors of Estimate Sum of Squares	d.f.
1	8	0.828**	0.000284	0.0086	7
2	8	0.360	0.000484	0.0066	7
3	8	0.237	0.001667	0.0025	7
Sub-Total	24	0.723**	0.00029	0.0180	23
4	8	0.737	0.000254	0.0077	7
5	8	0.077	0.000055	0.0016	7
6	8	0.500	0.002778	0.0013	7
Sub-Total	24	0.670**	0.00025	0.0111	23
7	8	-0.058	-0.000020	0.0180	7
8	8	0.325	0.000759	0.0096	7
9	8	-0.314	-0.0001559	0.0042	7
Sub-Total	24	-0.025	-0.000012	0.0333	23
Total	72	0.469**	0.000194	0.0712	71

[1] All regression coefficients are significantly greater than zero.

** Significant at $P \doteq .01$.

Table 29

Adjusted Means for Protein-Nitrogen in the Juice

Can Size	Processing Temperature °F.	Mean	Adjusted Mean
300 X 200	220	0.06	-0.01
	235	0.03	0.06
	250	0.02	0.06
300 X 308	220	0.06	-0.01
	235	0.03	0.06
	250	0.03	0.07
401 X 411	220	0.07	0.08
	235	0.06	0.06
	250	0.03	0.03

Table 30

Analysis of Covariance and Test of Significance of Adjusted Means for Protein-Nitrogen in the Juice

Source of Variation	Degrees of Freedom	Errors of Estimate: Sum of Squares	Errors of Estimate: Degrees of Freedom	Errors of Estimate: Mean Square	F Test
Between temperatures in can size 300 X 200					
Total	25	0.0249	24		
Treatments	2				
Error	23	0.0249	22	0.00078	
For test of significance of means		0.0069	2	0.00345	4.423*
Between temperatures in can size 300 X 308					
Total	25	0.0156	24		
Treatments	2				
Error	23	0.0111	22	0.00048	
For test of significance of means		0.0045	2	0.00225	4.688*
Between temperatures in can size 401 X 411					
Total	25	0.0360	24		
Treatments	2				
Error	23	0.0333	22	0.0014	
For test of significance of means		0.0027	2	0.00135	9.642**

* Significant at P = .05.
** Significant at P = .01.

expressed as a per cent of the same fraction for the homogenate, and the protein-nitrogen as grams per cent.

The total-nitrogen of the juice expressed as a per cent of the total-nitrogen of the homogenate was positively related to both processing time (b = 0.0124) and processing temperature. However, the differences between the adjusted means (showing the relationship with processing temperature) are statistically significant for can sizes 300 X 200 and 300 X 308 only. This analysis indicated that, as a result of time (also of processing temperature) the proportion of the total nitrogen in the juice increased.

The proportion of the trichloroacetic-acid-filtrate-nitrogen in the juice showed a positive relationship with processing time (b = 0.0083), and processing temperature in can sizes 300 X 308 and 401 X 411, although these relationships appeared to be negative in can size 300 X 200. However, the adjusted means are significantly different only for the first can size (300 X 200).

The tungstic-acid-filtrate-nitrogen of the juice was positively related to processing time (b = 0.0035) and appeared to be so related to the processing temperature, although the differences between the adjusted means are not statistically significant.

The protein-nitrogen showed a positive relationship to time and temperature in can sizes 300 X 200 and 300 X 308, and a negative relationship to these in can size 401 X 411. The differences between the adjusted means are significant for all can sizes.

c. Effect of processing on the pH of the meat and the per cent loss in weight of the meat during processing

Tables 31 to 33 present the data relative to the change in pH and the loss in weight of the meat (expressed juice) during processing.

It is evident that the meat became more alkaline as a result of processing. This is in agreement with the finding of Okuda (72) and Green (73). Those cases which show an increase in acidity are considered to be in error, probably the result of the faulty operation of the pH meter. The shift in pH suggests that thermal processing induced an extensive amount of hydrolysis or rearrangement of the proteins. In either case, the physical properties and consequent conditions of the meat would be greatly altered. This would be reflected in its texture.

There appeared to be no apparent trend in the weight loss during processing. However, since each value represents the combined effect of the three variables studied, trends are not always obvious.

Table 31

Effect of Processing in Can Size 300 X 200 on Change in pH and Per Cent Loss in Weight of Beef During Processing

Processing Temp. °F.	Processing Time Min.	pH		Per Cent Loss in Weight
		Raw Beef	Processed Beef	
220	247	5.21	6.05	35.63
		5.90	6.47	38.74
		5.74	6.14	38.04
	471	5.88	6.20	43.09
		5.83	6.13	41.39
		5.78	6.12	42.03
	641	6.55	6.78	37.97
		6.07	6.40	43.86
		6.03	6.30	42.86
235	72	5.90	6.03	38.80
		6.02	6.37	39.27
		6.70	6.86	37.04
	89	5.70	5.81	36.45
		--	5.90	40.80
		5.73	5.96	39.74
	124	5.70	6.62	39.74
		6.21	6.47	37.15
		5.82	6.02	37.04
250	47	5.74	5.93	39.86
		5.70	5.95	38.80
		5.68	5.93	39.04
	50	5.90	5.90	40.15
		5.97	6.08	42.86
		5.89	5.96	39.45
	53	--	6.05	40.74
		--	6.50	38.10
		6.10	6.28	35.63

* Values are average of three determinations.

Table 32

Effect of Processing in Can Size 300 X 308 on Change in pH and Per Cent Loss in Weight of Beef During Processing

Processing Temp. °F.	Processing Time Min.	pH Raw Beef	pH Processed Beef	Per Cent Loss in Weight
220	370	6.07	6.11	38.24
		6.20	6.25	39.39
		6.23	6.49	42.50
	497	5.87	6.08	43.15
		5.87	6.36	43.00
		5.83	5.99	40.56
	676	5.69	6.10	44.15
		5.91	6.44	43.21
		5.93	6.17	45.97
235	101	5.79	6.01	41.21
		5.57	5.87	40.12
		5.71	5.80	41.50
	118	6.10	6.06	40.83
		5.90	5.77	44.15
		5.94	5.92	40.98
	147	6.01	5.90	38.83
		5.81	5.97	41.74
		5.90	5.97	39.77
250	67	5.78	5.96	42.89
		5.80	6.04	41.86
		5.89	6.08	41.74
	70	5.68	5.90	40.10
		5.59	5.80	40.01
		5.53	5.82	42.97
	73	6.03	5.88	42.77
		6.00	5.83	43.56
		6.12	6.05	40.89

* Values are average of three determinations.

Table 33

Effect of Processing in Can Size 401 X 411 on Change in pH and Per Cent Loss in Weight of Beef During Processing

Processing Temp. °F.	Processing Time Min.	pH Raw Beef	pH Processed Beef	Per Cent Loss in Weight
220	414	5.89	6.25	40.86
		5.82	6.17	33.59
		5.89	5.98	42.83
	589	5.98	5.85	46.91
		6.01	5.81	43.33
		6.10	6.07	40.45
	724	5.70	6.19	45.74
		5.64	5.77	42.04
		5.73	5.90	40.86
235	163	6.19	6.46	39.59
		6.82	6.78	34.16
		6.17	6.32	40.11
	178	5.90	6.10	40.93
		6.17	6.20	42.76
		5.80	5.80	44.57
	199	6.02	5.99	43.07
		6.00	6.25	39.84
		6.39	6.28	43.09
250	112	6.42	6.51	40.47
		5.93	6.08	43.45
		6.10	6.18	41.55
	116	5.69	5.98	41.49
		5.87	6.20	42.83
		5.70	5.90	41.83
	123	5.78	6.08	40.24
		5.71	5.73	44.55
		5.76	6.01	43.23

* Values are average of three determinations.

The statistical evaluation, presented in Tables 34 to 36, shows a positive relationship for per cent weight loss relative to time and temperature in all can sizes. The adjusted means, however, are statistically different only in the second can size.

d. Intermediary-nitrogen in the homogenate and juice

Intermediary-nitrogen is a term that was used to describe that nitrogen fraction which is the difference between the trichloroacetic-acid-filtrate-nitrogen and the tungstic-acid-filtrate-nitrogen. It includes, as shown by Hiller and van Slyke (74), all protein decomposition products smaller in molecular size than true proteins and larger in size than the lower or smaller peptides and amino acids. The reason for studying this fraction was to attempt to determine whether or not the changes noted in the non-protein-nitrogen fractions in the homogenate and in the juice were the result of the breakdown of tissue proteins, the conversion of collagen to gelatin, or, possibly, both. In order to do this, it was necessary to show which fraction of the blend or juice contained the collagen hydrolysis product (gelatin). No data relative to the precipitating action of either trichloroacetic acid or tungstic on gelatin could be found in the literature, although it was suspected that

Table 34

Regression and Correlation Data for Per Cent Loss in Weight by Beef During Processing

Group	Degrees of Freedom	Correlation Coefficient	Regression Coefficient[1]	Errors of Estimate Sum of Squares	d.f.
1	8	0.652	0.0109	37.8564	7
2	8	-0.192	-0.0128	17.9480	7
3	8	-0.239	-0.1794	29.1974	7
Sub-Total	24	0.476	0.0104	89.2806	23
4	8	0.783**	0.0142	18.1470	7
5	8	-0.311	-0.0230	16.0655	7
6	8	0.081	0.0406	13.3989	7
Sub-Total	24	0.578**	0.0134	52.0598	23
7	8	0.454	0.0219	92.1032	7
8	8	0.525	0.1029	54.5575	7
9	8	0.264	0.0786	15.2888	7
Sub-Total	24	0.376	0.0141	178.4142	23
Total	72	0.447**	0.0123	321.2301	71

[1] All regression coefficients are significantly greater than zero.
** Significant at P = .01.

Table 35

Adjusted Means for Per Cent Loss in Weight by Beef During Processing

Can Size	Processing Temperature °F.	Mean Per Cent Loss	Adjusted Mean
300 X 200	220	40.40	37.74
	235	38.42	39.52
	250	39.40	40.97
300 X 308	220	42.24	38.50
	235	41.01	42.80
	250	41.87	44.09
401 X 411	220	41.85	37.83
	235	40.90	42.46
	250	42.18	44.63

Table 36

Analysis of Covariance and Test of Significance of Adjusted Means for Per Cent Loss in Weight by Beef During Processing

Source of Variation	Degrees of Freedom	Sum of Squares	Errors of Estimate Degrees of Freedom	Mean Square	F Test
Between temperatures in can size 300 X 200					
Total	25	104.0466	24		
Treatments	2				
Error	23	89.2806	22	3.8818	
For test of significance of means		14.7660	2	7.3830	1.902
Between temperatures in can size 300 X 308					
Total	25	73.6006	24		
Treatments	2				
Error	23	52.0598	22	2.2635	
For test of significance of means		21.5408	2	10.7704	4.758**
Between temperatures in can size 401 X 411					
Total	25	210.8179	24		
Treatments	2				
Error	23	178.4124	22	7.7571	
For test of significance of means		32.4055	2	16.2028	2.089

** Significant at P = .01.

gelatin would be insoluble in tungstic acid. Therefore, the following experiments were carried out to determine whether or not gelatin is precipitated by trichloroacetic, and whether or not there is a critical concentration for precipitation.

In the first experiment aqueous solutions of various gelatin concentrations were used. The precipitations and nitrogen determinations were the same as previously described. The results are presented in Table 37.

Table 37

Recovery of Gelatin* from Aqueous Solutions

Concentration of Gelatin Solution %	Gelatin Recovered in: Trichloroacetic Acid Filtrate % Recovery	Tungstic Acid Filtrate % Recovery
0.25	100.00	10.00
0.50	100.00	5.00
0.75	100.00	4.17
1.00	100.00	3.13

* Obtained from Central Chemical Co., Chicago, Ill.

It is evident that gelatin is not precipitated from aqueous solution (varying in concentration from 0.25% to 1.00%) by the action of 5 per cent trichloroacetic acid

since all of the gelatin in each solution was found in this filtrate. The amount precipitated by tungstic acid appears to be dependent upon the concentration of the solution, although the amount precipitated is not considered negligible. This experiment shows one striking fact, the difference in the amounts recovered in the two filtrates (100 per cent against what is considered to be none). This suggests that the gelatin which is derived from collagen as the result of processing is measured in the trichloroacetic-acid-filtrate-nitrogen fraction.

It then had to be determined whether or not the same recoveries could be made in the presence of the homogenate or the juice of the processed meat. To do this, the experiment was repeated except that the gelatin was dissolved in a suspension of the homogenate and, also, in a solution of the juice. The test solutions were prepared by adding equal portions of a 5 per cent suspension of the homogenate and a 5 per cent solution of the juice to aqueous gelatin solutions varying in concentration from 0.50 per cent to 1.00 per cent. The results are presented in Table 38.

It is evident that the gelatin was not precipitated by the trichloroacetic acid, and that only a small portion of it is soluble in tungstic acid when in the presence of either the homogenate or the juice of canned meat. These

results indicate that the gelatin formed during processing would be measured as a part of the intermediary-nitrogen fraction.

Table 38

Recovery of Gelatin When Dissolved in the Blend and Juice from Processed Meat

Concentration of Gelatin Solution Added to Equal Portion of 5 Per Cent Suspension of Homogenate	Concentration of Gelatin Solution Added to Equal Portion of 5 Per Cent Solution of Juice	% Recovery in TCA*	% Recovery in TA**
1.00		98.65	6.76
0.75		97.27	7.60
0.50		98.30	8.20
	1.00	99.36	9.37
	0.75	99.18	12.03
	0.50	99.83	16.05

* Trichloroacetic Acid Filtrate.
** Tungstic Acid Filtrate.

These results also suggest that the "mushy" texture in canned beef must be in part the result of chemical changes other than the conversion of collagen to gelatin during processing. Prudent (79) found the average collagen-nitrogen content of the biceps femoris of an eight-year-old dairy cow (grading Canner-Cutter) to be 4.60 per cent of the total nitrogen. If it is assumed that this value represents the

average collagen-nitrogen content of the beef used in this investigation, then the conversion of collagen to gelatin cannot account for all of the differences noted in the trichloroacetic-acid-filtrate-nitrogen fraction between the raw and processed meat. Therefore, those differences, over and above those which can be accounted for by the presence of gelatin in this fraction, must be due to the degradation of other proteins.

This contention is supported qualitatively by the data presented in Table 39. These data show the histological ratings for the change in the collagenous tissue due to processing. The histological sections were prepared by embedding small pieces of raw and processed meat in paraffin, cutting both longitudinal and cross sections, and staining with Van Giesen's stain. Samples of the processed meat were obtained from both the outside and the center of the piece, and the changes in these sections were averaged to obtain the change for the entire can. Six replicates were made on each can.

These data show that it is possible to account for the increase in trichloroacetic-acid-filtrate-nitrogen by the conversion of collagen to gelatin only in a few cases (assuming the collagen-nitrogen of the raw meat to be 4.60 per cent of the total-nitrogen). In some of the cases, the

change in this fraction was much greater than could be explained by this conversion even though it had been a complete one.

Table 39

Change in Collagenous Tissue Due to Processing

Can Size	Processing Temp. °F.	Processing Time Min.	Total Nitrogen Gm. %	Increase in TCA* Nitrogen Gm. %	Increase as % Total-N	Average Collagen Change[1]
300 X 200	220	247	3.64	0.19	5.22	1
		641	3.81	0.31	8.14	-
	235	72	4.75	0.07	1.87	-
		124	4.02	0.20	4.98	1
	250	47	3.62	0.01	0.28	4
		53	4.16	0.08	1.93	-
300 X 308	220	370	3.69	0.24	6.50	7
		676	3.82	0.16	4.19	6
	235	101	3.88	0.13	3.35	4
		147	3.67	0.14	3.82	3
	250	67	3.74	0.12	4.38	1
		73	3.60	0.13	3.61	3
401 X 411	220	414	3.82	0.16	4.19	6
		724	3.60	0.23	6.39	7
	235	163	3.67	0.14	3.82	3
		199	3.80	0.20	5.26	4
	250	112	3.63	0.24	6.61	4
		123	3.59	0.01	2.78	5

* Trichloroacetic Acid Filtrate

[1]

Rating	Change
1	nil
3	less than half
5	more than half
7	complete

IV. DISCUSSION OF RESULTS

The data presented furnish evidence that chemical changes which may occur during thermal processing, determined by the fractionation of nitrogen, may be responsible for the "mushy" texture in canned beef. However, there appears to be little direct relationship between the processing variables studied and the chemical changes which may have occurred.

It was assumed that proteolytic changes in beef, similar to those observed in the aging of meat, which occur during the initial stages of heat processing prior to the inactivation of the enzymes by heat, together with hydrolytic changes in the meat proteins, are responsible for the texture defect. It was also assumed that the hydrolytic changes are related, in some manner, to processing conditions.

The autolytic studies on chicken breast muscle, as well as those on beef, suggested that proteolytic changes observed during the aging of meat may not be directly responsible for the resulting tenderization. However, autolysis may cause certain minute changes in the chemical constitution of the proteins which are reflected in extensive alterations in their physical properties and consequently conditions. The autolysis studies did suggest that proteolysis may be in part responsible for the texture defect.

The results of the statistical analyses indicated that a relationship exists between the chemical changes which were observed and some of the processing variables studied. In general, there was a positive relationship between processing time and the change in the trichloroacetic-acid-filtrate-nitrogen fraction in the homogenate and juice (although occasionally a negative relationship was shown). The same relationship was observed in the tungstic-acid-filtrate-nitrogen fraction in the homogenate and juice, the total-nitrogen of the juice, the protein-nitrogen in the juice, and per cent loss in weight of the beef during processing. The positive relationship with time indicates that a decomposition of protein occurs, the extent of which is dependent upon the length of the process.

The relationship with processing temperature is less definite. In some instances it is positive, in others, negative. In those instances where a trend was indicated, the changes were not always significant. Thus, it is difficult to evaluate the effect of this variable.

The effect of size of the piece of meat cannot be evaluated since it was necessary to analyze the data for each can size separately.

The decomposition of proteins, indicated by the relationship of the changes with time, is supported by other

observations. The most obvious is the increase in trichloroacetic-acid-filtrate-nitrogen in the homogenate and juice as a result of processing. The increase in this fraction for all can sizes amounted to 28 per cent. Since there was no increase in the tungstic-acid-filtrate-nitrogen, the only conclusion that can be reached is that a degradation of the proteins to molecules of intermediate size occurred. This is supported by the increase in the total-nitrogen of the juice, and the shift observed in pH (indicating extensive hydrolysis or rearrangement of proteins). The conversion of collagen to gelatin cannot account for all of this degradation (shown by the gelatin recovery experiments), since, in many instances, the change in nitrogen was greater than that which could have been the result of this conversion.

The effect of proteolysis on the texture has not been definitely established. However, it has been established that proteolysis during the initial stages of heat processing is possible. This could have resulted in minute chemical changes in the constitution of the proteins which are reflected in an extensive alteration of the physical state. This alteration in the physical state could be a contributing factor to the "mushy" texture.

V. SUMMARY

The chemical changes which may occur in beef proteins during thermal processing and their effect on the texture of the canned meat were studied in this investigation. The relationship between the extent of these chemical changes and certain processing variables was also studied.

The method used to determine these changes involved a fractionation of nitrogen into that which is soluble in trichloroacetic acid and in tungstic acid. The processing variables studied were the size of the piece of meat canned, the processing temperature, and the processing time.

Evidence for the following conclusions has been presented:

1. Proteolysis in aging meat is not directly responsible for tenderization, but may bring about minute changes in the chemical constitution of the muscle proteins which cause an extensive alteration in the physical state of the proteins.

2. Tenderization during storage may be the result of a physical alteration in muscle proteins.

3. Proteolytic changes in beef, similar to those observed in aging meat, which may occur during the initial stages of heat processing, appear to be responsible in part

for the "mushy" texture of canned beef. Although not directly responsible for this texture defect, they may bring about a physical alteration in the meat proteins by a change in chemical constitution. This physical alteration may be in part responsible for the "mushy" texture.

4. The texture defect cannot be explained *in toto* by the conversion of collagen to gelatin, since the extent of increase in the trichloroacetic-acid-filtrate-nitrogen as a result of processing cannot be entirely explained by this conversion. This increase is interpreted to indicate the degradation of other muscle proteins.

5. The changes during processing in the nitrogen fractions are related only to the length of processing time. No definite relationship with processing temperature could be established. The relationship with the size of the piece of meat could not be evaluated.

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